

Use of HTRF technology to support pre-clinical biomarker studies for the development of antibodies in oncology

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Are our biotherapeutics hitting our target ?

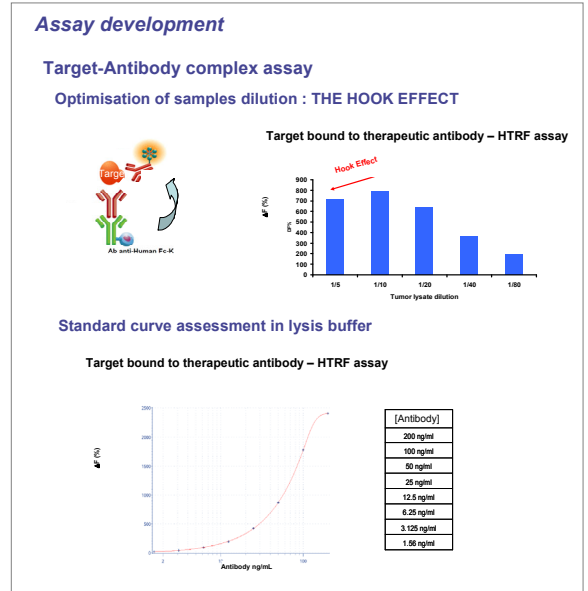
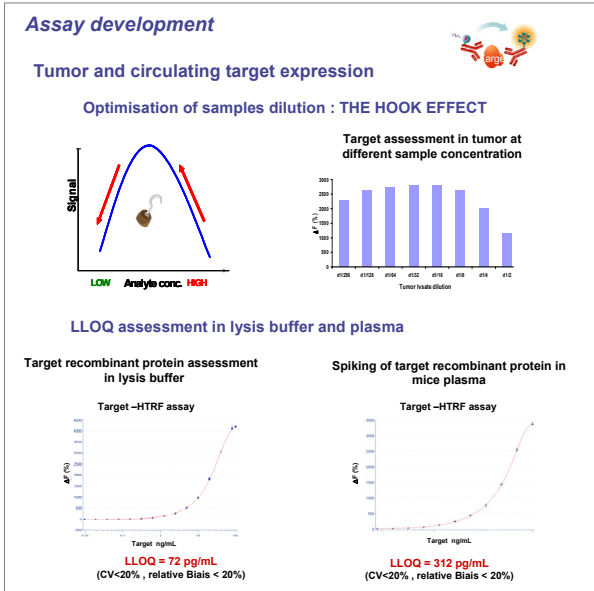
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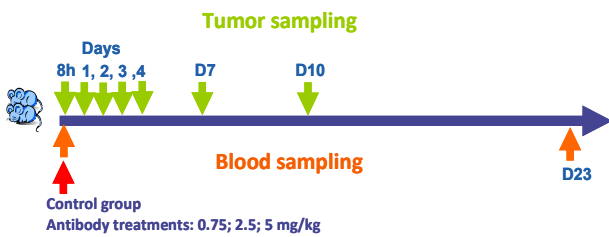
Abstract

As part of the development of antibodies targeting proteins involved in the oncogenesis process, the biomarker support is key. Indeed, one should be confident that when injected in tumor-bearing mice, our biotherapeutic is effectively reaching the tumor and binds to its target.

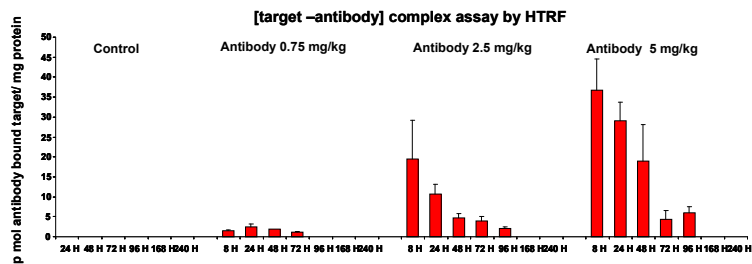
In this study, we will show how HTRF technology has been used to characterize the target expression in the tumor as well as in blood (shed target) at baseline. These assays have been also used to monitor both antibody targeting to the tumor and its effect on circulating target level in blood



Study design in colon Patient Derived Xenograft (PDX) model

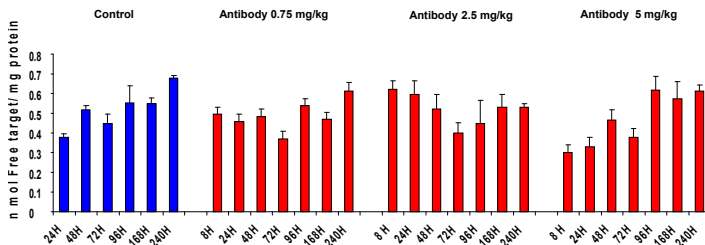


Therapeutic antibody is reaching the tumor

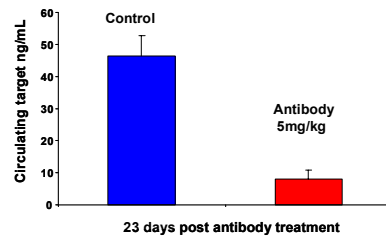


Target tumor expression is not affected by the treatment

Target expression assessment in colon PDX model by HTRF assay



Circulating Target level in blood is modulated by antibody's treatment



CONCLUSION: The HTRF technology enabled biomarker studies for the development of biotherapeutics in Oncology. Our biomarkers studies showed that :

- antibody reached the tumor in a time-and dose-dependent manner.
- antibody treatment decreased circulating target level and had not impact on target expression in tumor,

These data confirmed the Mechanism of Action of our biotherapeutic and suggested potential candidate for Pharmacodynamic(PD) biomarkers in our early clinical phases (circulating target level).

Methods:

Tumor expression and blood circulating target level: At indicated time point post treatment, mice were euthanized, tumors were resected and processed for lysis and biomarkers determination.

Levels of free target expression were determined by commercial HTRF target assay after dilution of samples and normalized by total protein concentration (BCA Protein Assay).

For circulating target level, plasma were collected at designed time point post treatment and directly analyzed using HTRF target kit assay.

[Target-antibody] complex assay: Tumor lysates were diluted in phosphate buffer and incubated with an anti human Fc-K (donnor) and an anti target –D2 (acceptor) antibodies. Results were given as pmol of antibody bound to the target by mg of total protein.