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

Are our biotherapeutics hitting our target?

Attanot F., Nicolazzi C., Chiron M., Henry C.
Sanofi oncology, 13 quai Jules Guesde 94403 Vitry-sur-Seine, France (mailing to florence.attanot@sanofi.com)

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 target tumor expression is not affected by the treatment

A1

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.

<table>
<thead>
<tr>
<th>Target-antibody complex assay by HTRF</th>
<th>[target–antibody] complex assay by HTRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Antibody 0.75 mg/kg</td>
</tr>
<tr>
<td>Antibody 2.5 mg/kg</td>
<td>Antibody 5 mg/kg</td>
</tr>
<tr>
<td>Antibody 5 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

LLOQ assessment in lysis buffer and plasma

Study design in colon Patient Derived Xenograft (PDX) model

Therapeutic antibody is reaching the tumor

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