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Biologics Done Right

Case Study

Method Transfer and Optimization of a Flow Cytometry Method Following Best Practice in a Regulated Environment

Client: Large Pharma

Therapeutic Area: Immuno-oncology

Patients:
Adult subjects with advanced solid tumors

Study Phase:
Multi-site Phase 1b/2

Services:
Flow Cytometry Method Transfer and Development

PROJECT AT A GLANCE

A global pharmaceutical company contracted Eurofins Bioanalytical Services to develop and then apply a Fit-for-Purpose validation assay utilizing frozen PBMC samples. This was to facilitate analysis of PBMC samples prepared at the clinical sites and shipped to Eurofins Bioanalytical Services for the analysis. The clinical study objectives were to determine the safety, tolerability, and recommended intradermal peptide vaccine in a combination therapy regimen with checkpoint inhibitors atezolizumab (anti-PD-L1 monoclonal antibody), or nivolumab (anti-PD-1 monoclonal antibody), in adult subjects with advanced solid tumors. The project required Eurofins Bioanalytical Services to identify and quantitate the phenotype and extent of activation in treated PBMC samples. The primary cells of interest within the PBMC samples were the WT1-restricted $CD8^+$ cytotoxic T lymphocytes (CTL). Using a multi-parameter immuno-phenotyping panel and MHC-restricted tetramer reagents of activation state, a number of different HLA-A haplotype $CD8^+$ T cells of varying MHC could be identified. In addition to the identification/quantitation, we were also asked to generate WT1-restricted $CD8^+$ CTL, using client-provided vaccine peptides to serve as Process QCs for both method qualification assays and sample analysis QCs.

SITUATION

This assay employed a 7-color flow cytometry method that required extensive modification and development, performed in close collaboration with the client. The original client assay used frozen PBMC samples and it was generating variable results at their current CRO partner.

On review this was considered to be due to the lack of suitable process QCs to objectively monitor the day-to-day analysis in original method.

The project objectives were defined as:

- Immunophenotyping the $CD8^+$ T cells of interest and detecting upregulation in the $tetramer^+$ signal.
- Starting with the original qualified method, optimize the assay by working closely with the client.
- Generate assay QCs using the client-provided vaccine peptides and an in vitro maturation protocol.

Sample Analysis at the

RIGHT TIME & RIGHT PLACE

to satisfy data delivery requirements

CHALLENGES

De Novo Generation of WT1 CD8⁺ CTL Cells:

- The peptide vaccine was in very limited supply (10 mgs) and required that the tissue culture used to generate the WT1-restricted CD8⁺ CTLs be done correctly the first time. Note: A particular challenge in addition to the limited supply of the vaccine peptides was the 11-day in vitro culture protocol requiring multiple reagents and growth factors added at various time points to produce the CD8⁺ CTL cells /assay QCs.
- The client defined the percentage of PBMCs transformed though peptide stimulus to WT1 CTL cells. The set percentage (% WT1 restricted CD8⁺ CTLs) were used to categorize the outcome as either a 'positive' or a 'failed' attempt.

Analysis Templates and Instrument Set Up:

- The client provided Eurofins Bioanalytical Services with WT1-restricted CD8⁺ CTLs with three different HLA-A haplotypes, to aid in assay optimization and creation of suitable instrument-specific analysis templates. The proportion of tetramer⁺ CTLs in these samples was established by the client prior to shipping to Eurofins Bioanalytical Services. For the secondary analysis of these samples, it was required to develop an assay and analysis templates able of obtain values within ±30% of sponsor's pre-defined ranges.
- Limited drug quantities, pre-defined performance specifications and a short timeframe required a pragmatic approach to assay transfer and validation experiments. There was a need to **"Get it right the first time!"**.

SOLUTIONS

- Successfully generated a large volume of WT1-restricted CD8⁺ CTLs, using client-provided vaccine peptides. This improved on the original method by enabling performance monitoring of the daily analyses, trending assessments, and other aspects requested by the client.
- We designed and implemented efficient multi-panel analysis templates, and stayed within the client's pre defined ±30% range.
- Our collaboration was successful and reduced time and costs. We met client expectations by providing excellence in the laboratory execution and clear communication throughout the project.
- Using Eurofins Bioanalytical Services expertise and attention to detail redesigned the in vitro maturation protocol, including changes in reagents and maturation times during the 11-day culture period.
- Followed the current scientific best practice recommendations [1,2,3] and where applicable regulatory guidelines [4].

References

1. O'Hara paper 2011
2. Du paper 2009
3. Future Science, Bioanalysis, Vol.9(16), White Paper
4. FDA BMV 2018

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About Eurofins Bioanalytical Services

Over 20 years of industry-leading global **Scientific Expertise** supporting the widest breadth of Biologics' clinical trials with PK/TK, ADA, NAb and Biomarker assays and sample analyses.

Versatile Performance and Project Management Excellence to adapt to a client's specific needs. Clinical or preclinical, regulated or non-regulated, assay development, qualification or validation; we custom design our support to match the client's program.

State-of-the art laboratory facilities in St. Charles, MO, USA providing **Global Reach and Capacity** to address clients' needs while simultaneously offering regionally-based solutions.

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