Assessment of Pharmacodynamic Effects of Immuno-Oncology Agents in Cynomolgus Monkeys using High-Content Gene Expression Profiling

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**BACKGROUND**

Nanostring nCounter analysis system performs a highly multiplexed, digital quantification of nearly 800 genes in a single reaction. This is achieved with the help of reporter codesets, which are color-coded “barcodes” specific for each gene. Workflow consists of three major steps: 1) Hybridization 2) Purification 3) Digital imaging. In the hybridization step, sample material is mixed with excess codeset and the codeset hybridizes to mRNA target in solution. Purification is carried out robotically with the help of prep station which removes excess codeset and immobilizes codeset/RNA complexes in the nCounter cartridge for data collection. CCD capture technology is used for data collection and digital images are processed by the digital analyzer and reporter probes counts are tabulated for data analysis using Nanostring’s nSolver analysis software.

**OBJECTIVE**

To assess pharmacodynamic (PD) effects of immunomodulatory agents in cynomolgus monkeys (Macaca fascicularis) using a high-content gene expression platform (nCounter Non-Human Primate (NHP) Immunology Panel, NanoString) that covers 20 immunologically relevant pathways.

**RESULTS**

**DIFFERENTIAL GENE PROFILING WITH WBL**

Figure 1. A) A linear curve was obtained with positive controls. Only background subtraction was done for data analysis, negative controls were not used. B) Expression of housekeeping genes were similar across all the samples

**Differential expression of genes**

Blood was collected from naïve cynomolgus monkeys, before and at various timepoints after intravenous administration of experimental immunotherapies.

nCounter analysis was focused on select timepoints/samples along with respective controls based on previously generated data from QuantiGene plex (affymetrix), a comparable gene expression platform.

Two different sample types, purified RNA from peripheral blood cells and whole blood lysates (WBL), were evaluated in this platform. RNA and WBL demonstrated comparable performance, hence only data from WBL is discussed here.

**Method**

- Blood was collected from naïve cynomolgus monkeys, before and at various timepoints after intravenous administration of experimental immunotherapies.
- nCounter analysis was focused on select timepoints/samples along with respective controls based on previously generated data from QuantiGene plex (affymetrix), a comparable gene expression platform.
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**CANONICAL PATHWAYS ALTERED WITH IMMUNOTHERAPY AGENT**

Figure 2. WBL was used as an input material and differentially expressed genes with two fold change and p<0.05 are highlighted in the volcano plot. Genes which are shifted towards left of the volcano plot are downregulated and shifted towards right are upregulated.

**CONCLUSIONS**

- The data indicate that the Nanostring nCounter gene expression platform was capable of detecting immune-related PD effects of immunomodulatory agents in cynomolgus monkeys using both RNA and whole blood lysates.
- High-content molecular platforms such as nCounter can significantly enhance PD assessment and broaden understanding of immune-related changes in NHP studies which may facilitate informative decision making and translational PK/PD modeling.