

## **Methodology and Criteria for High-circulating Myeloid-derived Suppressor Cells (MDSCs) in Part H**

Blood levels of monocytic(m)MDSCs were determined using a Clinical Laboratory Improvement Amendments (CLIA)-validated flow cytometry assay developed by a commercial laboratory, Seramatrix (Carlsbad, CA). The Seramatrix flow cytometry assay is designed to identify the CD14+ myeloid subset of cells that are negative for lineage markers of T, B, and NK cells and negative or low for human leukocyte antigen DR (HLA-DR) expression using a modification of an algorithm first described by Kitano et al (1). The sample requirement is 5 mL of whole blood collected in a Cyto-Chex BCT (Streck, NE) tube containing a stabilizing reagent. Samples are shipped at ambient temperature directly to the Seramatrix facility where they are stained with a panel of antibodies for flow cytometric analysis on the FACSVerse Flow Cytometer with quality control and results analysis supported by FloJo (Treestar) and R Console R Foundation for Statistical Computing software.

The mMDSC phenotype has been shown to be stable under these conditions for 5-7 days. Baseline levels of mMDSCs were determined using the CLIA certified Seramatrix lab located in the United States. The cutoff of 20.5% for MDSC high/low was chosen based on historical data from a range of solid tumor types analyzed by Seramatrix. Patients with high MDSCs ( $\geq 20.5\%$ ) were enrolled into Part H based on the prediction that approximately half of the patients screened would fall into this range. In a small metastatic melanoma study using this same MDSC assay, patients with baseline MDSCs  $< 20.5\%$  correlated positively with response to ipilimumab (2). We hypothesized that eganelisib in combination with checkpoint therapy would benefit patients with high baseline MDSCs ( $\geq 20.5\%$ ).

### References:

1. Kitano S, Postow MA, Ziegler CG, Kuk D, Panageas KS, Cortez C, et al. Computational algorithm-driven evaluation of monocytic myeloid-derived suppressor cell frequency for prediction of clinical outcomes. *Cancer Immunol Res.* 2014;2(8):812-21.
2. Kitano S, Postow MA, Cortez C, Rasalan T, Gallardo HF, Panageas K, et al. Myeloid-derived suppressor cell quantity prior to treatment with ipilimumab at 10mg/kg to predict for overall survival in patients with metastatic melanoma. *Journal of Clinical Oncology.* 2012;30(15\_suppl):2518-.