PPD Laboratories now offers assays for quantitation and detection of pembrolizumab, bevacizumab and ipilimumab, extensively used and clinically successful immuno-oncology therapies. These assays are available for clinical analysis for protocols that include pembrolizumab, bevacizumab and ipilimumab as primary or co-medications.

PEMBROLIZUMAB
Pembrolizumab is a humanized IgG4κ anti-PD-1 monoclonal antibody (MAb) used as a checkpoint inhibitor in the treatment of various cancers and is known commercially as Keytruda®. Pembrolizumab is an immunotherapy approved for treatment in melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, classical Hodgkin lymphoma, primary mediastinal large B-cell lymphoma, urothelial carcinoma, microsatellite instability-high cancer, gastric cancer, cervical cancer, hepatocellular carcinoma, Merkel cell carcinoma, and renal cell carcinoma. It is used as both first- and second-line treatment for certain indications and in combination with other therapeutics and is currently part of over 1000 clinical trials. PPD has developed and validated PK and ADA assays for pembrolizumab for sponsors to use in support of their clinical trials.

PK ASSAY:
PPD Laboratories has developed and validated a quantitative PK assay to measure pembrolizumab in human serum using a sandwich ELISA. The quantitation range is 30.0 to 2560 ng/mL, with no hook effect and dilutional linearity demonstrated from 1,250,000 ng/mL to 69.4 ng/mL. There is no hemolytic or lipemic interference at maximum physiological levels and selectivity has been demonstrated in normal human serum. Total error for the assay was less than 20% across the assay range. Target or co-medication interference and specific disease state selectivity testing can be performed upon request.

ADA ASSAY:
PPD Laboratories has developed and validated an ADA assay for anti-pembrolizumab antibody detection in human serum using electrochemiluminescence. A traditional 3-tiered approach is used with an additive screening assay cut point factor of 13.9, a confirmatory cut point of 14.6% and an additive titer cut point of 24.9. Drug tolerance is demonstrated at 100 ng/mL anti-pembrolizumab Ab in the presence of 125 µg/mL Pembrolizumab. Screening and confirmatory assay sensitivity is less than 36 pg/mL. A calculated 1% LPC is set at 47 pg/mL. Hemolytic interference was identified at ADA levels < 1 ng/mL; no lipemic interference was identified. Target interference was detected at levels perceived to be clinically irrelevant.

Both assays have been validated per GLP and in accordance with applicable PPD SOPs and current regulatory guidance.
**BEVACIZUMAB**

Bevacizumab is a humanized mouse recombinant MAb that inhibits VEGF-A, blocking angiogenesis and is known commercially as Avastin®. Bevacizumab is approved for treatment of metastatic colorectal cancer, non-squamous non-small cell lung cancer, recurrent glioblastoma, metastatic renal cell carcinoma, persistent, recurrent or metastatic cervical cancer, epithelial ovarian, fallopian tube or primary peritoneal cancer and wet-AMD and other diabetic eye diseases. It is used as both first- and second-line treatment for certain indications and in combination with other therapeutics.

**PK ASSAY:**

PPD Laboratories has developed and validated a quantitative PK assay to measure bevacizumab in human serum using electrochemiluminescence. The assay quantitation range is 500 to 16,000 ng/mL, with a hook effect observed above 125,000 ng/mL and dilutional linearity acceptable from 1,250,000 ng/mL to 1250 ng/mL (up to a 1000-fold dilution). Hemolytic interference was observed at low drug concentrations (≤1200 ng/mL) in the presence of high levels of hemolysis (5% hemolytic matrix). There was no lipemic interference at maximum physiological levels and selectivity has been demonstrated in normal human serum. Precision and accuracy was less than 15% across the range of the assay. Target or co-medication interference and specific disease state selectivity testing can be performed upon request.

This assay has been validated per GLP and in accordance with applicable PPD SOPs and current regulatory guidance.

**IPILIMUMAB**

Ipilimumab is a fully human anti-human CTLA-4 monoclonal antibody (MAb) used as a checkpoint inhibitor of various cancers and is known commercially as Yervoy®. Ipilimumab is an immunotherapy that is used to treat multiple cancers, first being approved for the treatment of melanoma. The indications have since been expanded, approving it for use to reduce risk of skin scanner relapse after surgery. Most recently, it has been approved in combination with low dose Nivolumab for the treatment of microsatellite mismatch repair (MSI-H/dMMR) metastatic colorectal cancer.

**PK ASSAY:**

PPD Laboratories has developed and validated a quantitative PK assay to measure ipilimumab in human plasma containing dipotassium EDTA using liquid chromatography with tandem mass spectrometric (LC/MS/MS) detection. An immunoaffinity approach with magnetic beads coated with Protein G was used to enrich the MAb from human plasma. The antibody being too large for practical direct quantitative analysis using LC/MS/MS technology, it is subjected to proteolysis with trypsin, following standard protein denaturation, reduction and alkylation processing steps. As a result, characteristic peptide fragments originating from the anitbody are produced, these peptides were quantified as surrogates for determinign concentrations. The method is applicable to the quantitation of Ipilimumab within a nominal range of 1.00 to 100 μg/mL adn requires a 30.0-μL human plasma aliquot. Both intra- and inter-assay precision and accuracy were within 25% for the LLOQ and within 20% for all other levels.

This assay has been validated per GLP and in accordance with applicable PPD SOPs and current regulatory guidance.