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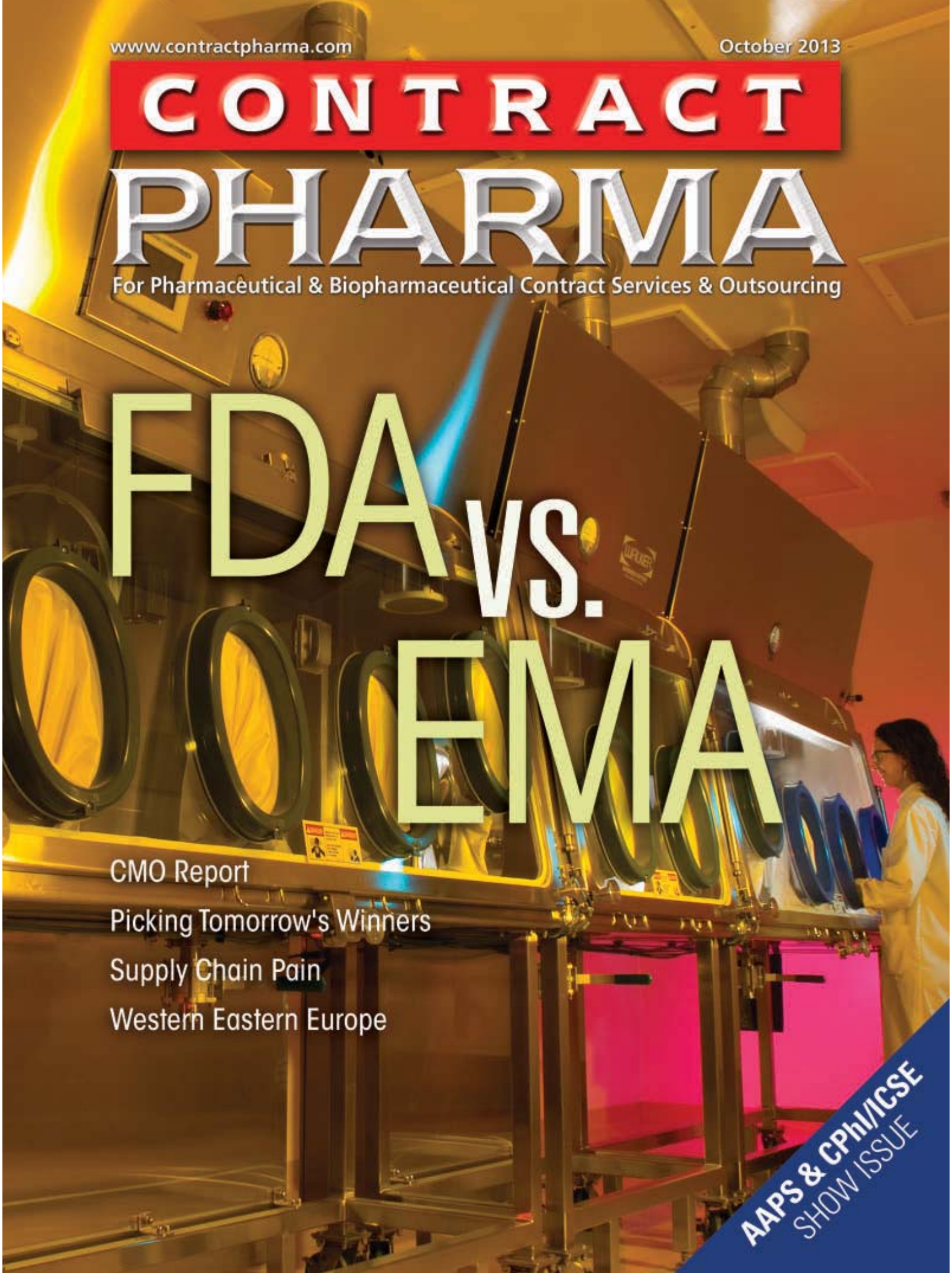

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Flow Cytometry

Vellalore Kakkanaiah of PPD on biomarker development

PPD INC. HAS AN EXTENSIVE STRATEGY to support biomarker development and testing for its CRO clients. I talked with **Vellalore Kakkanaiah** of PPD's bioanalytical laboratory about how the company employs flow cytometry in its development of biomarker assays for client studies, and where he sees client demand coming from in future.

—GYR

Contract Pharma: *What is the role of flow cytometry in drug development?*
Vellalore Kakkanaiah: Flow cytometry assays can be used in every stage of drug development, from preclinical to patient stratification and selection. In recent years, a major shift has been occurring in the pharma industry toward the development of large molecule drugs. For example, more than two dozen monoclonal antibody (MAb) drugs are currently marketed in the U.S. and EU for the treatment of various diseases including cancer, autoimmune diseases and asthma. It has been reported that nearly 350 new antibody-based drug candidates are entering clinical trials. This trend is predicted to continue.

Biomarkers are playing an increasingly pivotal role in drug development, so the industry understands the need to identify translational biomarkers to aid in demonstrating the safety and efficacy of drug candidates. Ultimately, these advances should reduce the time and cost of developing these drugs. Flow cytometry plays an important role in the analysis of cellular biomarkers.

CP: *Where does it fit in with traditional bioanalytical methods?*
VK: Flow cytometry is an ideal tool to evaluate discovery and pharmacodynamic biomarkers. For example, we developed an assay at our Richmond, VA bioanalytical lab to monitor the level of cell surface target engagement for an antibody drug development program. The drug is an antibody and binds to the receptor sites on the cell surface. We wanted to measure how many of the receptors are occupied by the antibody drug compound and how long it stays in the system. We use the dye-conjugated antibody drug to measure the available receptors. The measured fluorescence intensity is inversely proportional to the concentrations of occupied receptors. In this case, the fluorescence intensity can be converted to molecules of equivalent soluble fluorochrome (MESF) using Quantum MESF beads with known fluorescence values, and the number of antibodies bound per cell can be extrapolated.

CP: *How does PPD differentiate its flow cytometry capabilities from other CROs or internal labs?*
VK: PPD offers flow cytometry assays at both our bioanalytical and central lab facilities. The flow cytometry lab located at our Richmond facility operates as part of our immunochemistry department in accordance with the principles of Good Laboratory Practices (GLP) and applicable regulatory agency guidances. Flow cytometry assays are also available at our central labs in Belgium, Singapore and the U.S., where they mainly operate under CLIA regulations (42CFR493) and guidances from CLSI and CAP.

We also maintain a flow cytometry lab at our Phase I clinic in Austin, Texas, to process the samples from Phase I studies where the stability of the samples is too short for off-site shipping.

CP: *Why do clients outsource flow cytometry instead of building capacity internally?*
VK: The main reason for outsourcing is the lack of resources to analyze a large number of samples. For example, it is easier to process preclinical samples using the in-house laboratory assays, but the in-house lab may not have the instrumentation and personnel to support Phase I to IV studies. Also, when the data are included for the regulatory submission, outsourcing to a reputable laboratory to obtain compliant data may be necessary.

CP: *What's your experience in handling these assays in your lab?*
VK: At our Richmond lab, we've processed more than 8,000 samples, mostly from Phase I and some Phase II studies. Most of the assays involve immunophenotyping of major cell populations, like T cells, CD4 and CD8 T cells, B cells, NK cells, basophils, eosinophils, platelets and their subsets. We also measure intracellular phosphorylated proteins on activated cells and activation markers on cultured cells.

Biographical Note

Vellalore Kakkanaiah, Ph.D., is associate director in the immunochemistry department at PPD's bioanalytical lab in Richmond, VA. He also manages the flow cytometry services at PPD's Phase I clinic in Austin, Texas. DR. Kakkanaiah has co-authored numerous publications in experimental and clinical medicine. For more information on the company's flow cytometry services, contact ppdinfo@ppdi.com.

At the Phase I clinic, we performed the target engagement assay for a drug compound on CD192 and CD195 receptors. These samples had been processed on-site at the Phase I clinic due to stability limitations of the receptors.

CP: *How long does it take to validate an assay?*
VK: It depends on the assay type. For a client-transferred exploratory biomarker assay, it can take two to four weeks, while for a secondary objective biomarker assay, it can take four to eight weeks for complete validation.

The validation time does not include the development of the assay, which is a separate effort that again depends on the nature of the assay.

CP: *How have regulatory and quality standards changed as flow cytometry has become more useful for drug development?*
VK: As recommended in the FDA draft guidance on the method validation for bioanalytical methods, we follow a fit-for-purpose approach for validating flow cytometry biomarker assays, and GLP-like processes for documentation. The level of validation of an assay depends on the intended purpose of the data generated. For exploratory biomarker assays, the assays will be partially validated primarily for precision and the data will be subjected to QC review only. For secondary or primary objective biomarker assays, the assays will be fully validated and the project and data will be subjected to QC and QA review.

CP: *Is there any interaction, in terms of sharing best practices, between the Phase I lab and the central lab flow cytometry operations?*
VK: When we set up the assays at the Phase I clinical lab, we organize the documentation in the same way we do at our bioanalytical lab in Richmond. Training is also conducted to mirror what we have at Richmond.

CP: *Do you use QC samples for assays?*
VK: Commercially prepared quality control samples are available for only a few assays. Since most of the flow cytometry assays are laboratory-developed assays, there are no QC samples for most of those assays. However, we were able to prepare multi-level QC samples for a receptor occupancy assay that we developed for an antibody-drug compound program.

CP: *How do you perform data analysis?*
VK: The flow cytometer is equipped with data acquisition and analysis software. There are instances where we analyze the raw data on stand-alone flow cytometry analysis software. An experienced scientist will review the cellular populations before exporting the data for further statistical analysis.

CP: *How difficult is it to keep your flow cytometers operating optimally?*
VK: We verify the performance of these instruments every day before analyzing samples. At the bioanalytical facility, our standard procedures describe the operation and maintenance of the instruments, which include daily, weekly, monthly and semi-annual maintenance schedules. The semi-annual procedure includes preventive maintenance, which is done by the vendor's engineer.

CP: *What are PPD's flow cytometry capabilities outside the U.S.?*
VK: Currently, we support assays on frozen samples collected and processed outside the U.S. Our central labs also support some of these assays at our international locations, but under different guidelines. Because some sponsors desire a bioanalytical "guidance-like" approach, PPD is in the process of implementing a plan to be able to transfer and perform selected flow cytometry assays outside the U.S. at our central labs using a flexible approach. In these cases, an assay validated at our bioanalytical lab will be transferred to the central lab, cross-validated and applied to studies according to our bioanalytical method and procedures, with data analysis and project management coordinated by the bioanalytical lab. ■