Flow cytometry and pharmacokinetic studies

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Biography: Vellalore Kakkanaiah, PhD serves as the director of the flow cytometry group, which is part of the Biomarker Laboratories at PPD Richmond, VA. His team is focused on the development of flow cytometry methods to support drug development. Recently, his team developed a simple whole blood assay to study the cellular kinetics of CAR-T cells after adoptive cell therapy in multiple myeloma patients. Prior to joining PPD, Dr Kakkanaiah was a senior scientist at SurroMed, Inc. where he developed micro volume laser scanning cytometry assays. He earned a doctorate in immunology from Madurai Kamaraj University in India and then served as a postdoctoral fellow at Virginia Tech. His postdoctoral training involved the first identification of mature CD4-CD8 T cells by flow cytometry from the thymus of an autoimmune mouse model. He was also a fellow of the Arthritis Foundation at the UNC-Chapel Hill.

Vellalore N Kakkanaiah speaks to the *International Journal of Pharmacokinetics* about his experience in developing flow cytometry assays for measuring cellular kinetics in adoptive cell therapies. Flow cytometry is new to bioanalysis and is being used for measuring the cellular kinetics of infused cells from adoptive cell therapy. Here, he discusses the challenges in developing flow cytometry methods for monitoring the infused CAR-T cells.

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Can you briefly summarize the conventional bioanalytical methods used for pharmacokinetic studies?

Conventionally, chromatographic assays (LC–MS/MS) are used for small-molecule therapeutics with molecular weights approximately 100–1000 Da. For large molecules, which includes antibodies (biologics) and antibody–drug conjugates, ligand-binding assays are predominantly used. Recently, hybrid LC–MS/MS methods have been successfully employed for large-molecule therapeutics including antibodies and antibody–drug conjugates.

Can you describe the key regulatory requirements & the guidelines for pharmacokinetic studies?

Established guidelines from the US FDA are available for bioanalytical method validation for PK studies [1]. The following bioanalytical parameters should be optimized for method validation; reference standards, critical reagents, calibration curve, quality control samples, selectivity and specificity, sensitivity, accuracy, precision, recovery and stability of the analyte in the matrix.

How does flow cytometry play a role in pharmacokinetic studies?

Flow cytometry is used to measure cellular kinetics or *in vivo* PK monitoring of infused cells in adoptive cell therapy, such as recently approved CAR-T cell therapies. Cellular kinetics differs from conventional PK of small and large molecules, where traditional PK components, like distribution, metabolism and excretions are not applicable to these ‘living drugs’, since they are capable of proliferation *in vivo* after infusion. Monitoring the expansion and persistence of these infused cells is useful for understanding adoptive T-cell therapies and to study its relationship to efficacy and safety.

What are the key challenges in the development of flow cytometry bioanalytical methods for pharmacokinetics?

The first challenge is procuring samples with transgene expressing cells for development of the method, as they are not present in the normal subject or patients. Therefore, transgene expressing cells need to be manufactured using normal healthy samples. Second, since flow cytometry methods use whole blood, bone marrow and CSF, these samples need to be analyzed within 1–2 days due to limited cell stability. The third challenge is the limited availability of commercial reagents against customized transgene products, so in most cases these reagents need to be prepared and well characterized. The fourth challenge is the related technology; flow cytometry is a highly complex system, which requires experienced individuals to perform sample and data analysis. The data is generated without a calibration curve and considered quasi-quantitative. Finally, there is no regulatory guidance for flow cytometry method validation and the methods are validated as fit-for-purpose validation.

What are the most common challenges of pharmacokinetic studies in modern-day laboratories & what solutions do you use to address these?

Regulatory guidelines for bioanalytical method validation are available for traditional PK analysis that uses ligand-binding assays and chromatographic methods. Since published regulatory guidances for newer technologies such as qPCR and flow cytometry are lacking, the methods are validated as fit-for-purpose using published white papers. These issues were discussed extensively in the 2019 13th Workshop on Recent Issues in Bioanalysis (WRIB) [2] and it was recommended to follow scientifically-led method development and validation strategies, with support from the MIQE guidance [3] for qPCR method validation and CLSI Guideline H62 for flow cytometry method validation (CLSI Guideline H62: validation of assays performed by flow cytometry [UNPUBLISHED & UNDER REVIEW]).

What emerging technologies are you particularly excited about, which are likely to change the future of pharmacokinetic studies?

The new emerging technologies focus on high sensitivity, multiplexing, high-throughput and digital readouts. Numerous instrumentations are available for each methodology, for example, spectral flow cytometer, droplet digital PCR, multiplexed hybrid LC–MS/MS, high-resolution mass spectrometry, Simoa® and Gyros immunoassays. Choosing the methodology depends on the analyte to be measured and each has its own advantage over other, for example, the multiplexed hybrid LC–MS/MS is a better platform for PK analysis on samples from combination therapies with antibodies, compared with ligand-binding assays.

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