TRANSLATING FLOW CYTOMETRY ASSAYS FROM THE BENCH TO BEDSIDE

Flow cytometry assays are widely used across preclinical drug development studies ranging from target identification through hit confirmation and lead optimization studies[1]. Depending on the modality, flow cytometry assays can also be an integral part of pharmacology and toxicology studies – for example, complex modalities such as gene modified cell therapies like CAR-T cells, and antibody-drug conjugates (ADCs) that target cell surface proteins require the use of flow cytometry assays in preclinical and clinical studies. CAR-T therapies have shown clinical success in hematological malignancies and autologous cell therapies such as Kymriah, Abecma and Yescarta to name a few, are being used in specific oncology patient populations[2]. Additionally, there is significant drug development activities to develop allogeneic CAR-T cell therapies that are more scalable for broader patient segments. Flow cytometry assays have multiple applications including the detection and quantitation of genetically modified CAR-T cells and the monitoring of the chimeric antigen receptor (CAR) expression levels as a biomarker for efficacy. Additionally, flow cytometry assays can be used to monitor downstream effects such as cytokine release and toxic side effects such as immunogenicity.

The key advantage of using ADCs is the targeted delivery of chemotherapies to tumor cells to maximize efficacy and minimize toxicity. The targeting antibody in an ADC bind to cell surface antigens that are preferentially expressed on tumor cells, triggering internalization of the ADC complex. Once the complex is internalized, the chemotherapeutic payload induces tumor cell death. Flow cytometry is a robust and quantitative method to measure target antigen levels on tumor cells and normal cells, and can also be used to monitor internalization efficiency and payload release if the ADC is tagged with a fluorescent reporter[1]. Since flow cytometry measures changes at the single cell level, the bystander effect of ADCs or the ability to kill neighboring tumor cells, can also be measured, which is an important efficacy readout[1].

It is clear that flow cytometry panels and assays will continue to be widely used in preclinical drug development of specific modalities Flow cytometry panels have another very critical benefit – there is high translational value to the clinic. Multiplexed flow cytometry panels that are developed for preclinical studies can be used in clinical studies to monitor disease progression, therapeutic efficacy and toxicity. One important development is the 510(k) clearance from the FDA for Beckman Coulter's DxFLEX Clinical Flow Cytometer, allowing the marketing of the flow cytometer for clinical studies[3]. Clinically approved instruments are required for running flow-based assays in the clinic and another requirement are standardized protocols to ensure minimal variability and several scientific groups are developing standard operating procedures (SOPs) to run clinical flow cytometry assays. Some of the key considerations for clinical flow assays include assay sensitivity and reproducibility, standardized data analysis and manageable sample processing and analysis workflows[4]. Flow assays generate large amounts of data and data analysis can be subjective -for example, gating to segment specific cell populations can vary depending on the lab, user, analysis tools etc. While subjective analysis is acceptable in the preclinical setting, the data analysis of clinical samples has to follow a reliable protocol with little to no variation between samples. Several groups are working on standardizing flow panel data via automation and the development of specific analysis protocols for each assay[5]. Flow cytometry focused companies are developing assay specific kits to minimize variability in sample processing and wet lab analysis as kitted solutions are a good way to standardize assays across multiple labs and operators. Currently, flow panels have limited use in the clinic but adoption is likely to increase as end-to-end clinical workflows are developed. Based on recent developments, it is clear that flow cytometry panels are likely going to continue to be standard assays for preclinical drug development and specific panels will likely translate to clinical trials to monitor therapeutic efficacy and toxic side effects.

References:

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