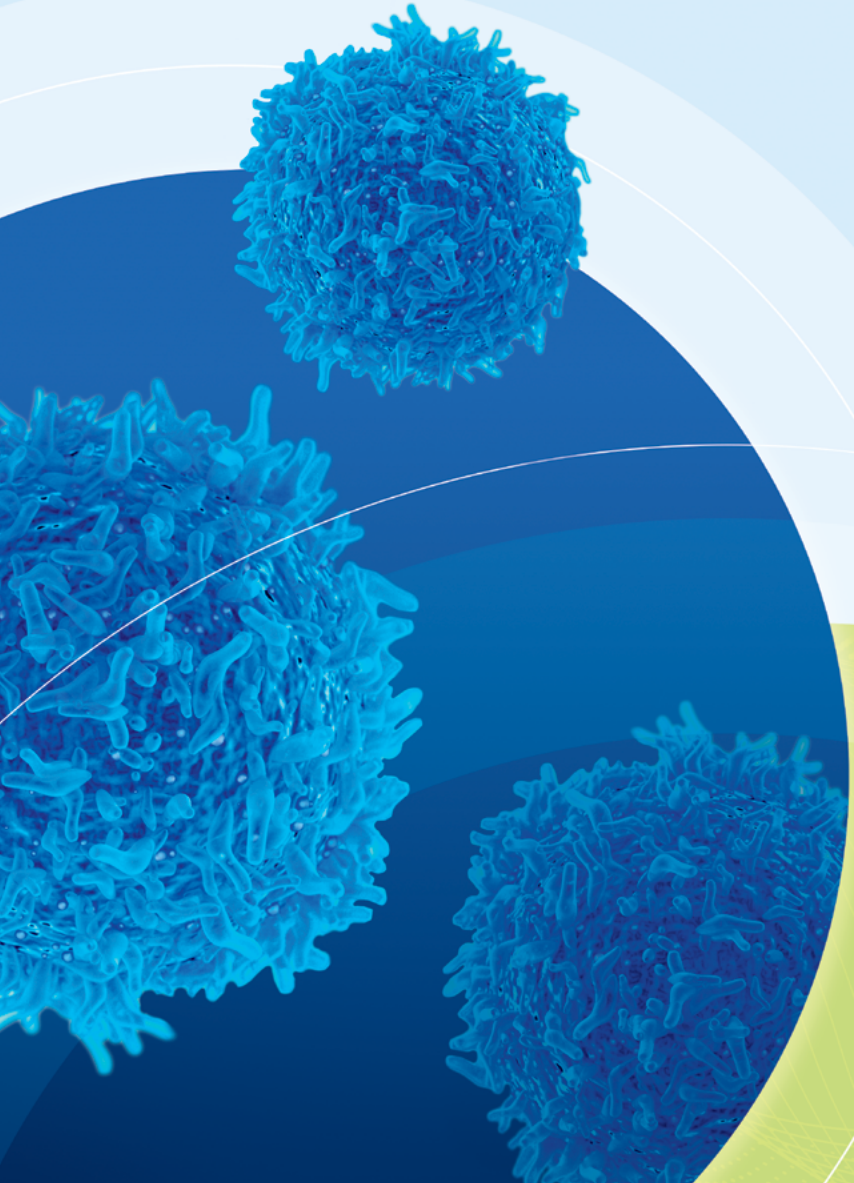
White Paper

FLOW CYTOMETRY

Assessments In Clinical Trials:

A Provider's Perspective

Nicholas Jones, Global Flow Cytometry Lead NeoGenomics Pharma Services



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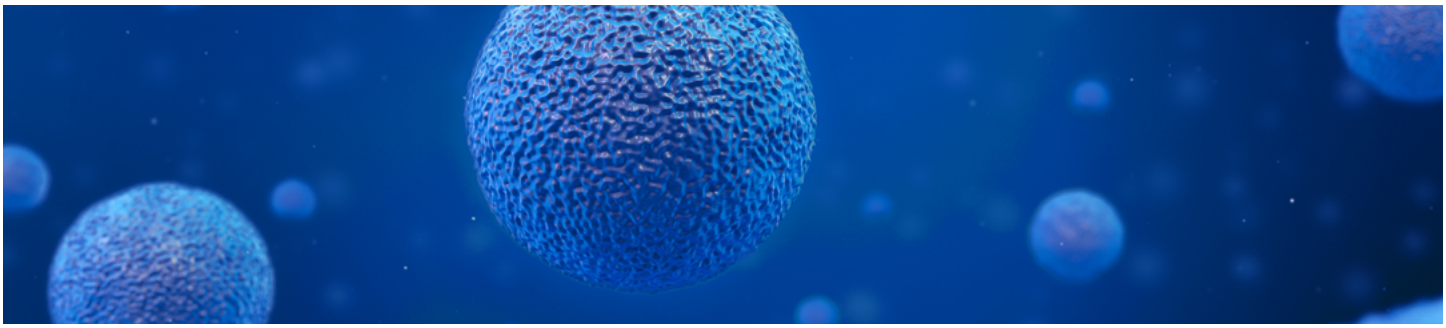
There are hundreds of clinical trials conducted by the Pharma industry in which flow cytometry is used to produce data that serves as an indicator of therapeutic drug or vaccine performance.

In some studies, these assays are considered to be standard assessments that are designed for the purposes of immunophenotyping or immune profiling, for which the data is mostly informational. However, in other circumstances the flow cytometry assays are specifically designed to determine drug targeted effects and this data is critical for evaluating therapeutic efficacy, selecting patient populations and/or directing patient treatment options. The most common designation applied to these types of assessments refers to them as "exploratory/RUO" or "Lab Developed Test (LDT)" assays, respectively. Each has very specific set of criteria that determine which path of validation a flow cytometry assay will follow.



Sponsor-Provider Discussion Topics

So what are the critical questions to ask and what information is needed when developing a new assessment? Who, between the client and the service provider, is responsible for making sure the right questions are asked and the right information is provided? It is generally assumed that this responsibility is shared, but the provider faces a unique challenge as they are ultimately responsible for the quality of the data as related to its intended use. Based on this, the provider needs to address specific questions in order to obtain information that can be used to make decisions related to performance of the assay, how the data is reviewed and finally how the data is reported. Due to potential negative consequences on study outcome, the commitment by the provider should always be to "do it right from the beginning". Providers should position themselves in the decision process by having consultative discussions with sponsors to obtain details that will impact the assay design and project plan.

Initial Questions for Sponsor-Provider Discussions

- What are timelines (assay development–implementation)?
- What are regulatory requirements (for the assay in the study)?
- What is the intended use of data?
- What is availability of intended use samples?
- Where is testing needed?

During the consultative process, there are important considerations that highlight the interaction between sponsor and provider. Discussions, in which the provider can establish known expertise and experience related to the current project and assay development, are essential to building confidence in their commitment to the project's success. These discussions set the stage for developing a specific program proposal for flow cytometry that addresses critical elements of the study. The final direction that an assay development proposal takes depends on the sponsor and provider having a clear and ongoing understanding of the study protocol prior to completion of the validation plan and initiation of the study.

Validation Criteria Determination

The details obtained through these discussions should provide sufficient guidance in order to finalize a project specific plan and initiate the development of a flow cytometry assay that meets all the performance criteria required for use in the study. Within this guidance, the provider should also have the information required to select the correct path of assay validation. There are specific criteria that define and differentiate validations that can be used to direct patient care (CLIA/LDT assays) and those that are for informational purposes only (RUO/Exploratory assays)

Further Discussion Points Related to Project Planning

- Level of assay validation required
- Type of therapeutic in development (drug type)
- Targeted disease indication
- Sample type for testing
- Instrumentation
- Reagent qualification
- Assay performance / sensitivity
- Testing and data consistency requirements
- Data analysis, review and reporting

Criteria Associated with CLIA / LDT Assays	Criteria Associated Exploratory Assays
Comprehensive analysis of patient progression	Comparative analysis across time points
Confirm disease at trial initiation	Confirm drug mechanism of action
Detect ongoing or recurring disease	PD/PK correlation
Enrollment criteria	Support dose selection
Full Pathology review and release	Predict potential response to drug

Both validation approaches have similar "basic criteria" that must be met regarding the development and validation process prior to implementation. These expected requirements define the backbone of any flow cytometry assessment and include specificity, precision, reproducibility and robustness. However, because of the clinical utility of an LDT assay, additional measures are required that extend beyond the criteria for exploratory flow cytometry assays. These criteria are critical in order to meet higher standards for quality and regulatory purposes. The acceptability of data by regulatory agencies for advancing the development of therapeutic drug programs rely heavily on fulfillment of these additional criteria.

Additional requirements for development of LDT flow cytometry

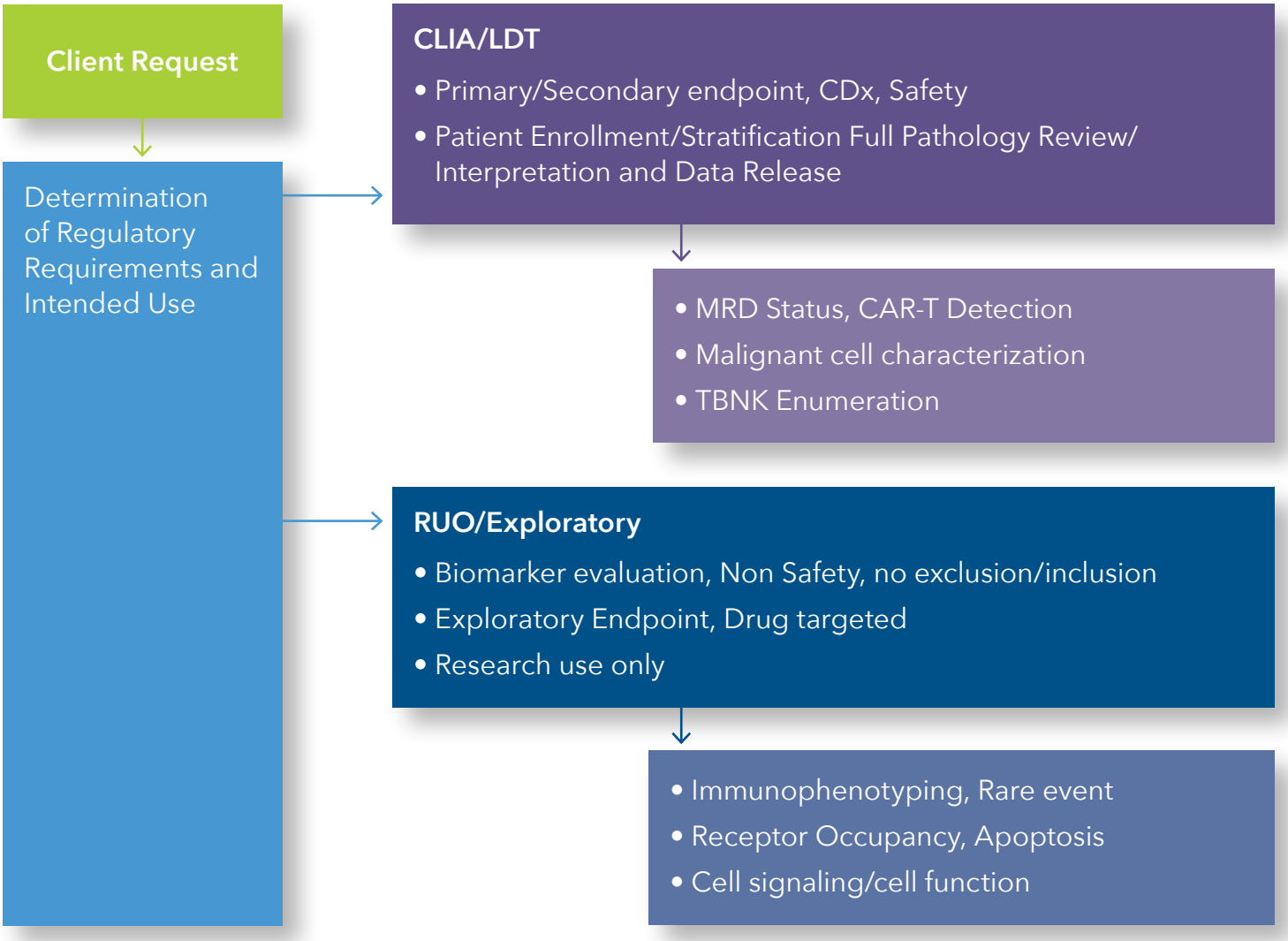
- Accuracy
- Lower limit of detection
- Limit of quantitation
- Linearity

Important Considerations for Decision Making

Discussions regarding flow cytometry assays in general, as well as descriptive plans for the development/validation of flow cytometry assessments, often focus on the number and type of markers that are employed. However, the upfront consultative process and information gathering are critical to establish the optimal assay design and utility of the markers employed.

Providers experienced with delivery both types of flow cytometry methods in a clinical trial setting can help guide sponsors to select the best developmental pathway for their study protocol. NeoGenomics has both experienced scientists and hematopathologists, who can provide input on study design.

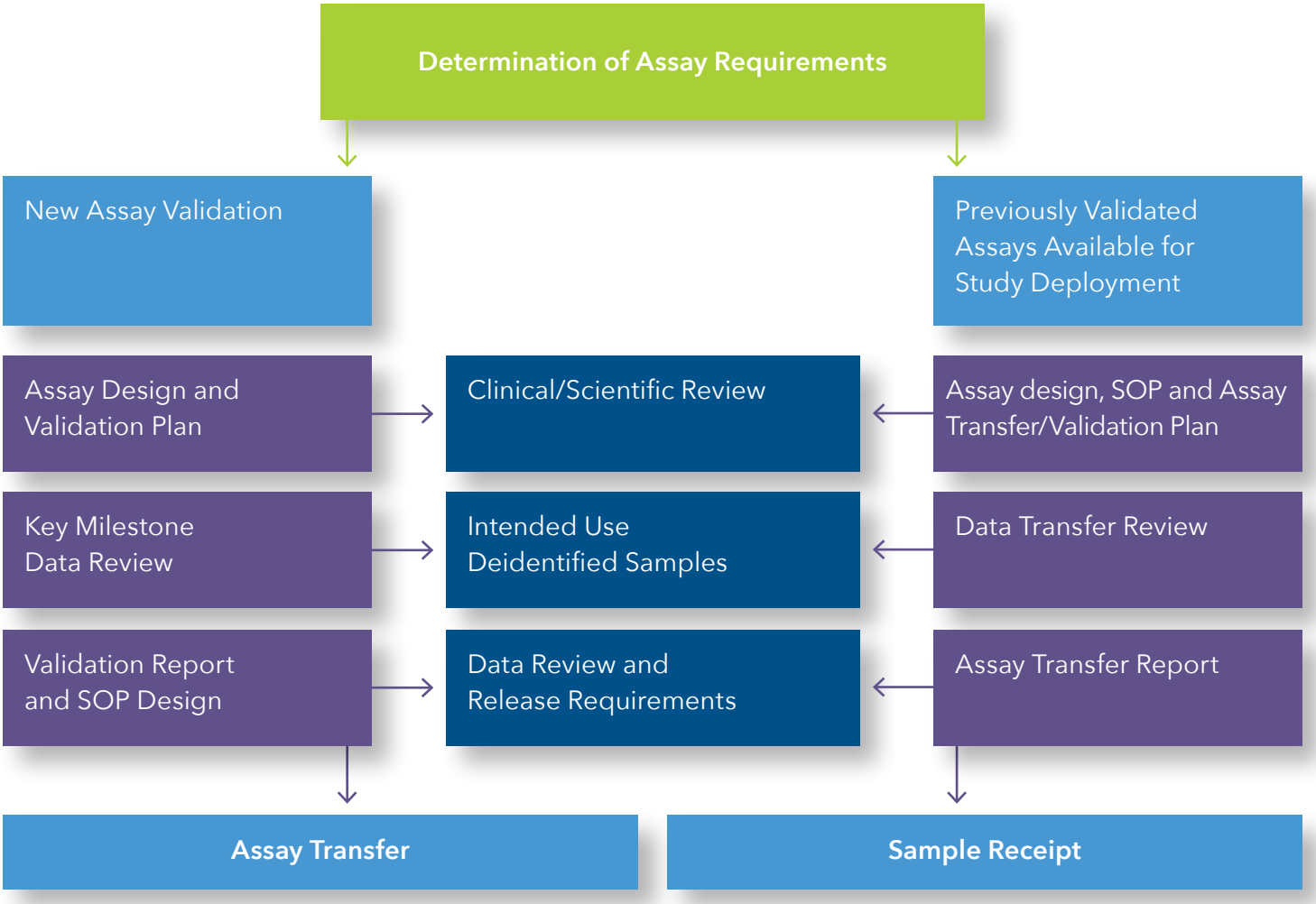
Decision Making for Flow Cytometry Assay Design and Validation



Assay Validation Process

After the path of assay development and validation is determined, there is a very deliberate process of checks and balances that reinforces the established stepwise procedure and includes continual sponsor interaction. There are two validation tracks that can be pursued. One includes complete development and validation by the provider, and the other involves a direct transfer of an "inhouse" validated flow cytometry assessment from sponsor to provider. Each track has specific stopping points at which the sponsor and provider can review progress before moving onto the next step of validation. It is important to ensure that during the course of assay development specific performance criteria are met. Stepwise review of the validation progress affords the sponsor and provider the opportunity to make changes and/or reassess the formulation of the flow cytometry assay to deliver qualitative or quantitative results. This deliberate process ensures that the data obtained is aligned with the study goals.

Deliberate and Collaborative Assay Validation Process



Assay Conversion from Exploratory to LDT

The initial decision to follow the development path for an exploratory assay is not self-limiting regarding its future use by the sponsor. During the course of a drug program as data is being evaluated, the established exploratory biomarker(s) may be shown to have predictive value on either drug activity or targeted disease parameters. This determination by the sponsor could elevate the utility of the flow cytometry assay to a level that informs the course of patient care or inclusion of specific patients in a study. In which case, it would be necessary to perform additional validation studies to transition the exploratory assay to an LDT assay. Such a transition would require intense scrutiny of the assay, its validation, and data by a knowledgeable team comprised of biomarker scientists, clinicians and regulatory personnel. In such cases, the team must thoroughly review the guidance for implementing flow cytometry predictive biomarkers into clinical trials. Prior to acceptance as a predictive biomarker assay, additional criteria that differentiate LDT from exploratory assays must be met. In other words, a revalidation with intended use samples and establishing data acceptance criteria is required to satisfy regulatory requirements.

In addition to these performance criteria, there are heightened processes and standards that are associated with delivering a predictive flow cytometry biomarker assay. Most are associated with clear and concise documentation that control each aspect of assay performance and data handling.

Additional Assay Performance Requirements

- Dedicated clinical expertise and consistent SOP
- Accepted instrumentation
- Advanced process for reagent selection and qualification
- Inclusion of relevant QC materials
- Strict consistency for data analysis and path review
- TAT for decision making

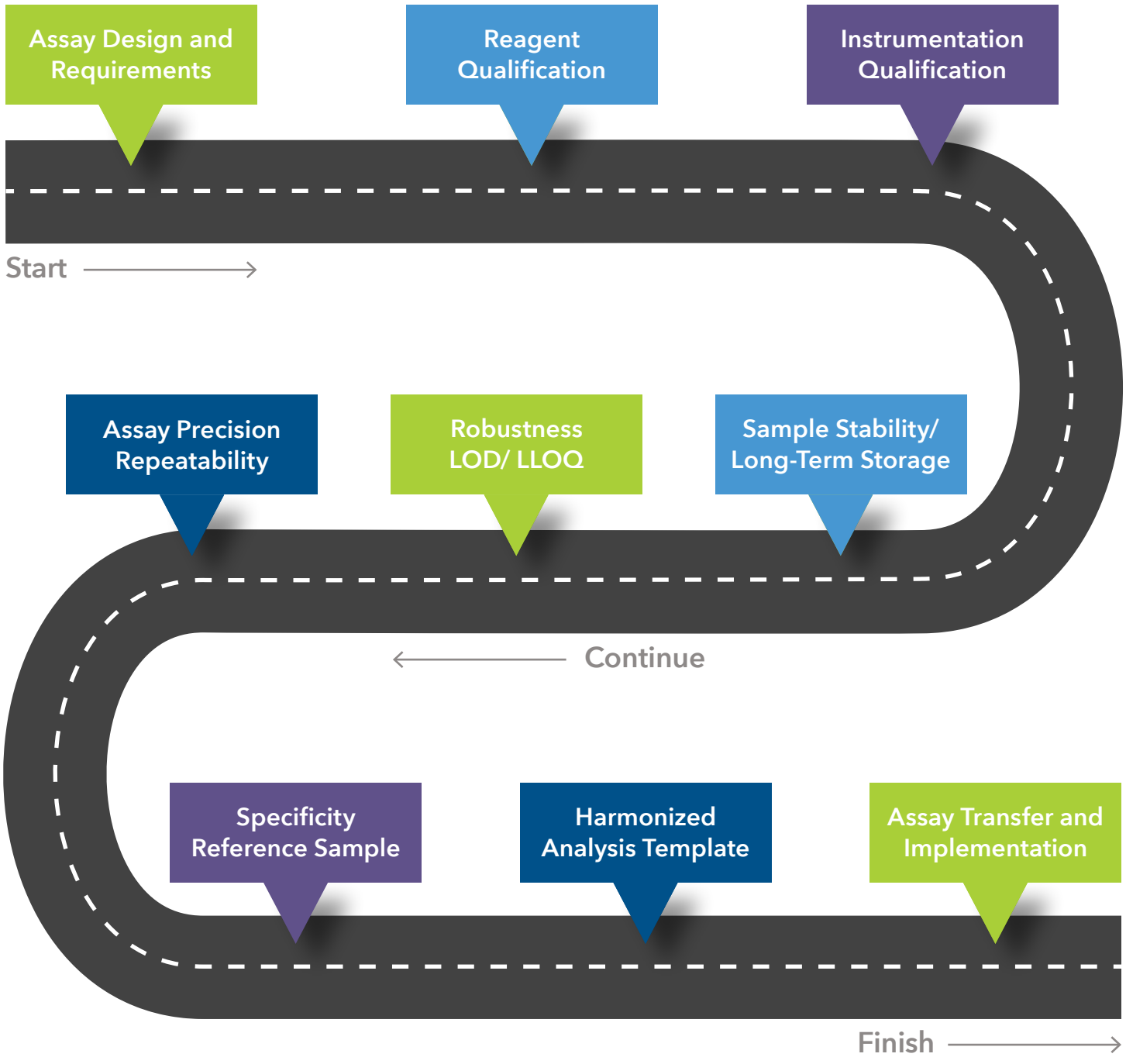
Process and Standards Needed to Deliver Predictive Flow Cytometry Biomarker Assays

Establishing a Successful Roadmap for Assay Validation

From Start to Finish, each assay has a unique beginning that then follows a determined roadmap for development, validation, transfer and implementation. The timeline for completion may vary with the complexity of the assay, its route of validation, or unexpected events (or delays) in the "winding road" process to completion.

Regardless, there are important stop points along the way that must be met prior to advancing to the next step. As mentioned earlier, sponsors are involved at multiple levels within the validation process to review and approve of data and/or assay performance before moving further down the road to completion.

Roadmap for Successful Assay Development



Differentiating LDT from RUO/Exploratory Assessments

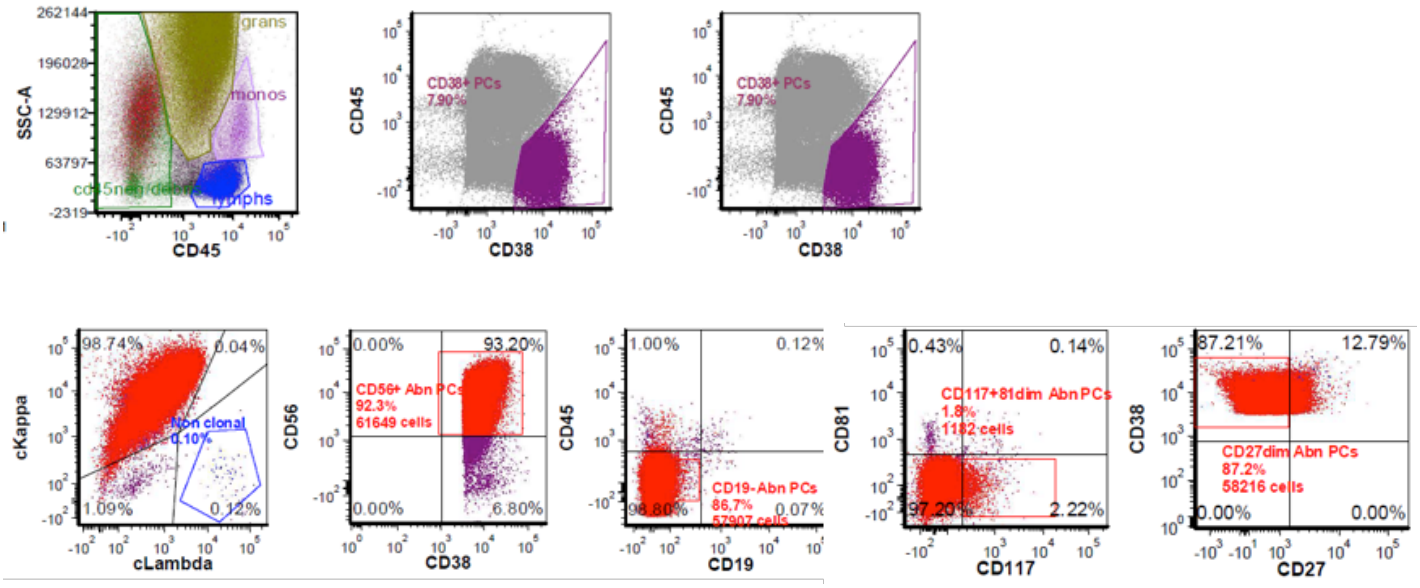
When data is produced and provided to the client, there are two significant differences in process between exploratory and LDT flow cytometry assays. The first important distinction is how the data is reviewed: For LDT assays, all data must be reviewed and signed off by a clinical hematopathologist. For RUO/exploratory assays, the data is reviewed and signed off by technical experts in flow cytometry that have been signed off on the specific SOP and analysis template. The second important difference is how the data is reported. For the LDT assays, the data is reported as a clinical interpretation with mention of the biomarker(s) expression in the assessment of relevance to patient status. For exploratory assays, the data is usually provided through a database results template as qualitative or quantitative values relative to the biomarker(s) detection or expression. These differences are illustrated in the following figures that exhibit the two assay types with representation of the data and different levels of reporting.

Example LDT Assay Type

MM MRD

- Development requires access to MM patient bone marrow
- 0.001% sensitivity
- 5 million events collected
- Single tube assay (10 color)

Fluorochrome	FITC	PE	PC 5.5	PE-Cy7	BV 421	BV510	BV 605	APC	APC-A700	APC-H7
MM MRD	**cKappa**	cLambda	**CD117**	CD19	**CD81**	**CD38**	**CD27**	**CD138**	**CD56**	**CD45**



Example LDT Assay Data Report

Diagnosis:
- Clonal plasma cells are identified:
%MRD of total nucleated cells: 0.002%
MRD count: 70

Percentages from CD38+ and CD138+ gate (70 events)
CD56: 45 events, 64.29%
CD117: 2 events, 2.86%
CD81: 12 events, 17.14%
CD27: 4 events, 5.71%
CD19: 13 events, 18.57%
Non Clonal: 2 events, 2.86%

Key Points

- Output includes Diagnosis and Interpretation
- Pathologist Review and Signoff
- Reported to clinical site investigator for potential patient treatment decisions in addition to sponsor

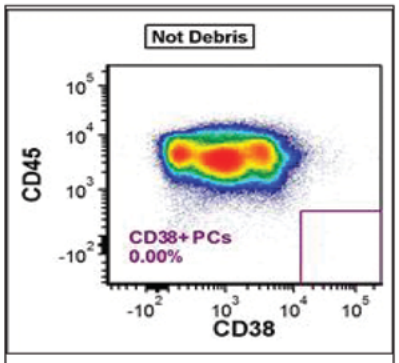
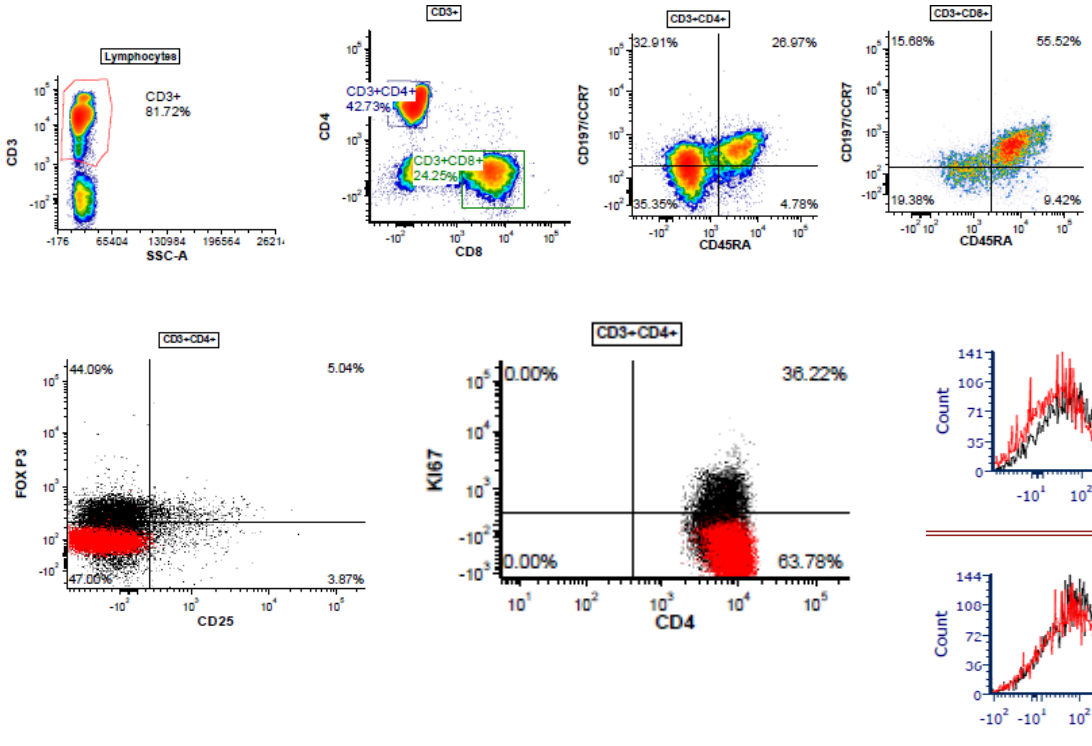


Fig 1 CD45vsCD38

Markers Performed:
CD19, CD27, CD38, CD45, CD56, CD81, CD117, CD138, cKappa, cLambda (10 Markers)

Example Exploratory (RUO) Assay Type

Lasers	Blue					Red			Violet						Ultraviolet	
Fluorochrome	FITC	PE	PE Dazzle	PE-Cy7	PerCP-Cy5.5	AF647	APC-R700	APC-Fire750	BV 421	BV510	BV 605	BV 650	BV 711	BV 785	BUV 395	BUV 525
T-Cell Profiling Assay	CD4	Blank	Blank	Blank		Blank	CD3	Blank	Blank	CD45RA	CCR7 (CD197)		Blank	Blank	CD8	
	CD4	CD137 (4-1BB)	TIGIT	PD-L1 (CD274)		Ki-67	CD3	PD-1 (CD279)	FoxP3	CD45RA	CCR7 (CD197)		CD25	LAG3 (CD223)	CD8	



Example Exploratory (RUO) Assay Data Report

Lymphocytes	9.48%
CD3+CD4+CD137 (4-1BB)+	0.15%
CD3+CD4+TIGIT+	5.45%
CD3+CD4+CD25+FoxP3+PD-L1+	4.55%
CD3+CD4+CD25+FoxP3+LAG3+	0.00%
CD3+CD8+CCR7+CD45RA+	15.06%
CD3+CD8+CCR7-CD45RA+	55.53%
CD3+CD8+CD137 (4-1BB)+	0.00%
CD3+CD8+Ki67+	15.41%
Cytotoxic T-cells CD3+CD8+	25.11%
CD3+CD4+CCR7+CD45RA-	2.27%

CD3+CD4+PD-1+	0.00%
CD3+CD4+LAG3+	0.86%
CD3+CD4+CD25+	7.47%
CD3+CD4+CD25+FoxP3+CD137+	2.27%
CD3+CD4+CD25+FoxP3+TIGIT+	15.15%
CD3+CD8+CCR7+CD45RA-	0.37%
CD3+CD8+PD-1+	0.00%
Helper T-cells CD3+CD4+	42.59%
CD3+CD8+TIGIT+	8.58%
CD3+CD4+CD25+FoxP3+ Treg	0.73%
CD3+CD4+CCR7-CD45RA-	67.47%

CD3+CD4+PD-L1+	2.89%
CD3+CD4+Ki67+	12.92%
CD3+CD4+CD25+FoxP3+PD-1+	0.00%
T-cells CD3+	71.38%
CD3+CD4+CD25+FoxP3+Ki67+	22.73%
CD3+CD8+CCR7-CD45RA-	29.04%
CD3+CD8+PD-L1+	4.64%
CD3+CD8+LAG3+	0.00%
CD3+CD8+CD25+	0.48%
CD3+CD4+CCR7+CD45RA+	5.64%
CD3+CD4+CCR7-CD45RA+	24.62%

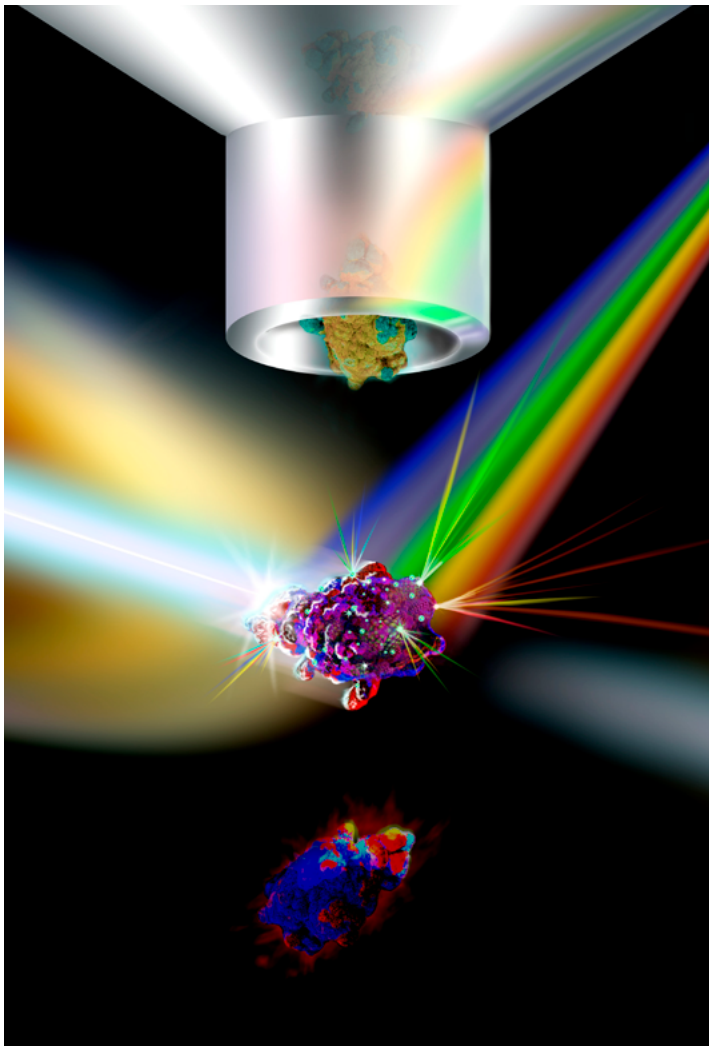
Key Points

- Output are data points / raw data
- No pathology review required
- Data is reported to sponsor or third party
- Results are for research use only and no patient treatment decisions can be made

Conclusion

From a provider's perspective, recognizing and understanding the differences in the development and performance of LDT vs RUO/Exploratory assessments, as well as the strict regulatory requirements for the intended use of data, is essential to the proper alignment of flow cytometry assays in clinical trials. In conclusion the following points are submitted.

- There is a significant increase in the use of LDT/ Exploratory flow assays in therapeutic drug development
- Very detailed sponsor-provider discussions are required for meeting regulatory requirements and goals of drug development programs
- It is critical to have exact information on the intended use of data
- Clinical, scientific, and quality should be represented in all discussions of assay validation and performance
- LDT assays have strict requirements for intended used samples during validation and appropriate QC material for assay performance in the trial
- Committed medical support is required for the review and reporting of LDT assay results
- Precise documentation including SOPs and change control is required to ensure data consistency across all testing locations



About NeoGenomics Pharma Services

NeoGenomics' Pharma Services unifies several innovative companies' scientific and medical leadership under one leading brand, offering one of the most comprehensive laboratory services menu available for biomarker testing supporting oncology clinical trials globally. We provide our clients with an unparalleled level of expertise, service, flexibility, and scalability. Additionally, we offer alternative business models and solutions across the continuum of development from pre-clinical research and development through commercialization.

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NEO GENOMICS

neogenomics.com

12701 Commonwealth Dr., Suite 9
Fort Myers, FL 33913
United States
Phone: 866.776.5907
Fax: 239.690.4237

31 Columbia
Aliso Viejo, CA 92656
United States
Phone: 800.720.4363
Fax: 949.425.5865

2131 Faraday Ave.,
Carlsbad, CA 92008
United States
Phone: 760.268.6200
Fax: 760.268.6201

2575 West Bellfort St., Suite 2001
Houston, TX 77054
United States
Phone: 800.720.4363/713.528.4363
Fax: 713.668.3565

A-One Business Center
Bâtiment A5, 2nd Floor
Z.A. La Pièce/Route de l'Etraz 1
1180 Rolle, Switzerland
Phone: +41 (0)21 721 06 00
Fax: +41 (0)21 826 00 73

NeoGenomics Singapore Pte Ltd
61 Science Park Road, #02-11,
Singapore 117525
Phone: +65 6591 5200