MRD Assessment in AML

Validation considerations for global clinical trial data

MetroFlow 2014

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Overview

- Biomarkers defined
- Fit-for-purpose validation
  - General
  - Considerations for Flow cytometry
- AML-MRD panel development
  - as a Biomarker in Clinical Trials
  - Challenges/solutions

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Definitions

- Biomarker
  - Biological marker, pharmacodynamic (PD) marker, pharmacological read-out
  - A characteristic that is measured and evaluated as an indicator of:
    - normal biologic processes
    - pathogenic processes
    - pharmacologic responses to a therapeutic intervention
- Clinical Endpoint
  - A characteristic or variable that measures how a patient feels, functions, or survives
- Surrogate Endpoint
  - A biomarker intended to substitute for a clinical endpoint
  - To predict clinical benefit or harm based on epidemiological, therapeutic, or pathophysiological scientific evidence

Clin Pharmcol Ther 69:89, 2001

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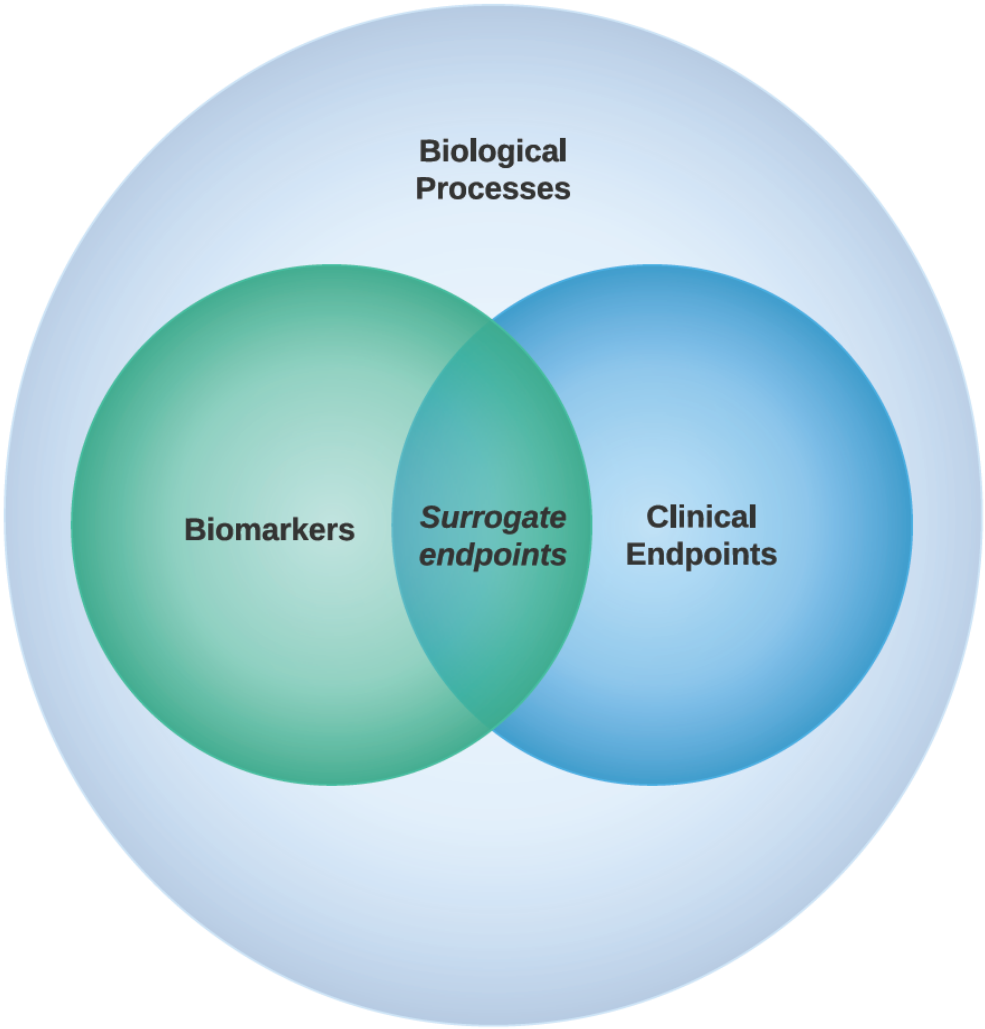
Biomarkers for Decision Making

•Target Validation	•Biomarkers that validate the importance of the target in humans disease
•Target/compound Interaction	•Biomarkers that define the chemical physical interaction of the compound with its target
•Pharmacodynamic Activity	•Biomarkers that define consequences of compound interaction with the target
•Patient Stratification	•Biomarkers that define likelihood of patients to response or not to the compound
•Adaptive Design	•Biomarkers that facilitate decision making during early clinical development via repeated measures that adjust study parameters

- ***Adapted from*** **Feuerstein G et al. Translational Medicine Perspectives. Am Drug Discovery 3(2), 2008**
- **Slide courtesy of Ole Vesterqvist**

Biomarkers vs. Surrogate Endpoints in Clinical Trials



A biomarker is

- measurable indications of a biological process or state
- must be *objective*, *quantifiable, reproducible*
- may or may not be clinically relevant
- may include physiological, biochemical, molecular interactions

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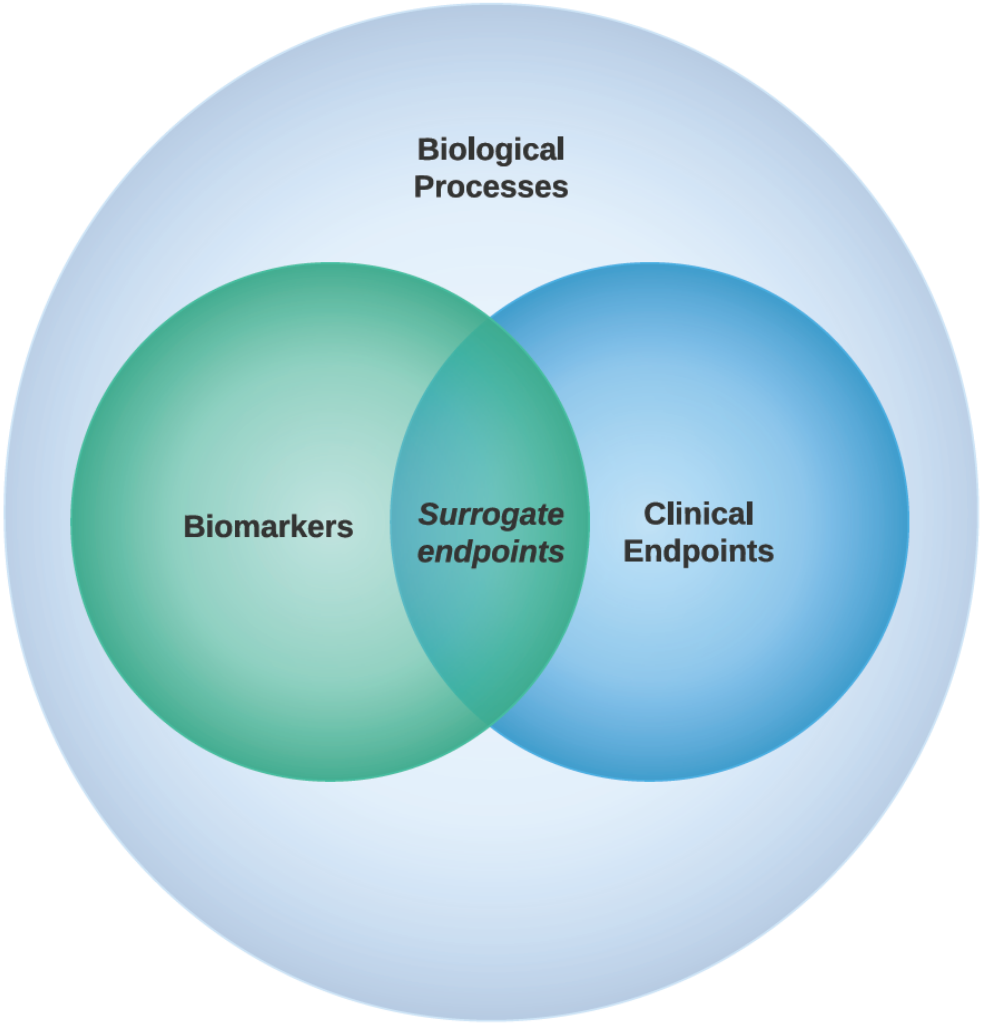
Biomarkers vs. Surrogate Endpoints in Clinical Trials



A surrogate endpoint is:

- biomarker subset with clinical relevance
- may stand-in for a clinical endpoint
- do not necessarily inform MoA or causation
- larger burden of proof is required, empirical data critical
- need to show *relevance* and *validity* or *ongoing evaluation* required (e.g. iterative approach)

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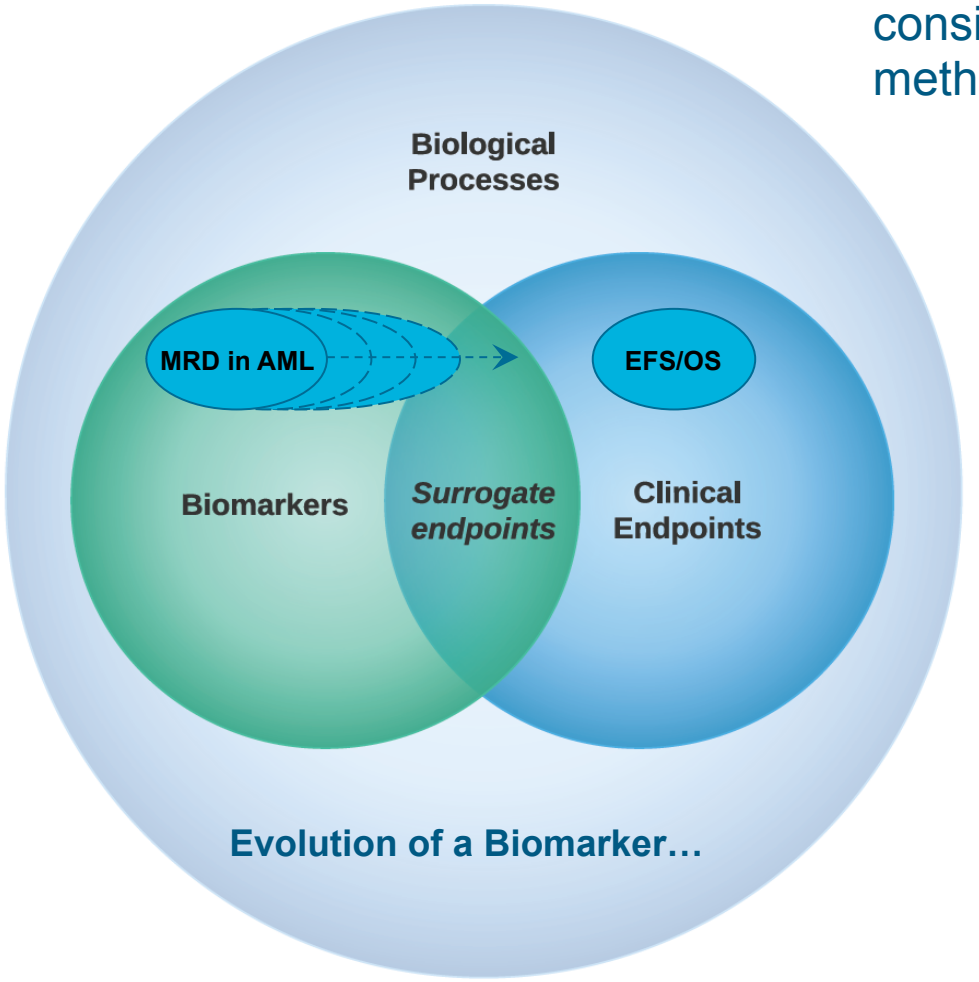
Defining Biomarker validation “space”



“When is a laboratory-developed test (LDT) considered a pharmacodynamic biomarker method?”

What is our validation space?

or

What is the purpose of our data?

for patient care and treatment → LDT

indicate response to a drug → PD Biomarker

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Flow Cytometry Validation

- The flexibility of flow cytometry allows for numerous potential applications but also adds to the complexity of assay development and validation.

- Flow cytometric methods are challenging to develop and validate.

- These challenges are related to:
  - Cellular analytes
  - Complex instrumentation
  - Reagents—light sensitive fluorochromes, mAb
  - Lack of cellular reference material

Fit-For-Purpose

Pharmaceutical Research, Vol. 22, No. 4, April 2005 (© 2005)
10.1007/s11095-005-2495-9

Conference Report

Method Validation and Measurement of Biomarkers in Nonclinical and Clinical Samples in Drug Development: A Conference Report

Jean W. Lee,[1,17] Russ S. Weiner,[2] Jeff M. Sailstad,[3] Ronald R. Bowsher,[4] Dean W. Knuth,[5] Peter J. O'Brien,[6] Jean L. Fourcroy,[7] Rakesh Dixit,[8] Lini Pandite,[9] Robert G. Pietrusko,[10] Holly D. Soares,[11] Valerie Quarmby,[12] Ole L. Vesterqvist,[2] David M. Potter,[11] James L. Witliff,[13] Herbert A. Fritche,[14] Timothy O'Leary,[15]

Pharmaceutical Research, Volume 23, No. 2, February 2006 (© 2006)
DOI: 10.1007/s11095-005-9045-3

Research Paper

Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement

Jean W. Lee,[1,16,17] Viswanath Devanarayan,[2] Yu Chen Barrett,[3] Russell Weiner,[3] John Allinson,[4] Scott Fountain,[5] Stephen Keller,[6] Ira Weinryb,[7] Marie Green,[8] Larry Duan,[9] James A. Rogers,[10] Robert Millham,[10] Peter J. O'Brien,[11] Jeff Sailstad,[12] Masood Khan,[13] Chad Ray,[14] and John A. Wagner[15]

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Fit-for-Purpose Approach in Flow Cytometry

Research paper

Recommendations for the validation of flow cytometric testing during drug development: I instrumentation

Cherie L. Green[a], Lynette Brown[b], Jennifer J. Stewart[b], Yuanxin Xu[c], Virginia Litwin[d], Thomas W. McCloskey[e]*

Recommendations for the validation of flow cytometric testing during drug development: II assays

*Denise M. O'Hara[a], Yuanxin Xu[b], Zhiyan Liang[c], Manjula P. Reddy[d], Dianna Y. Wu[e], Virginia Litwin[f],**

JIM, 363:104-119, 2011
JIM, 363:120-134, 2011

The Role of Biomarkers in Clinical Trials and The Fit-for-Purpose Method Validation Approach, V. Litwin and C. Green
http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm334772.htm

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Biomarker Validation Concerns

Limited experience interpreting biomarker data and an unclear regulatory climate

Recommendations might adhere too strictly to existing guidelines

GLP

- FDA guidelines for bioanalytical drug assays and safety monitoring

CAP/CLIA

- Diagnostic Assays

May not be suitable for many biomarker assays

Validation criteria could stifle creative biomarker solutions

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Biomarker Validation Questions

- What experiments should be performed?
- What data are necessary?
- What acceptance criteria are appropriate?

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Fit-For-Purpose Validation

Fit

- Biomarker data must be reliable and accurate data

Purpose

- Decision making during drug development

Fit-for-Purpose

- Analytical requirements should be stage-specific
- the intended use of the biomarker data
- the regulatory requirements associated with that use
- Practical, iterative approach

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Conclusions Fit-for-Purpose

"It is impossible to apply one set of rules to all assay platforms."

Unlike drug assays, novel biomarker assays are accompanied by unique analytical issues, in many cases ruling out the use of universal, strict validation guidelines

These issues include the common absence of suitable reference standards, the employment of unique analytical reagents and assay platforms, the presence of endogenous biomarkers in a sample, analyte heterogeneity, and a variety of disease-specific effects

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Fit-for-Purpose Approach in Flow Cytometry

Compared to other methodologies commonly used in drug development, such as plate-based ligand binding assays and mass spectrometry, flow cytometric methods can be more challenging to validate.

These challenges are related to the combination of cells, reagents, lack of cellular reference material and complex instrumentation.

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Flow Cytometry Validation

- The flexibility of flow cytometry allows for numerous potential applications but also adds to the complexity of assay development and validation.

- Flow cytometric methods are challenging to develop and validate.

- These challenges are related to:
  - Cellular analytes
  - Complex instrumentation
  - Reagents—light sensitive fluorochromes, mAb
  - Lack of cellular reference material

Getting Started with Validation

Prior to assay validation

- Assay Development Phase
- Panel Design
  - Antigen selection and fluorochrome pairing
  - Gating strategy and reportable results
- Instrument Setup and Compensation

Establish the validation pathway

- Type of Validation
  - IVD
  - RUO
  - LDT
  - Technology transfer
- Level of stringency
- Assay requirements
- Assay limitations

Validation Considerations

1. Assay Complexity

2. Validation Stringency & Regulatory Requirements

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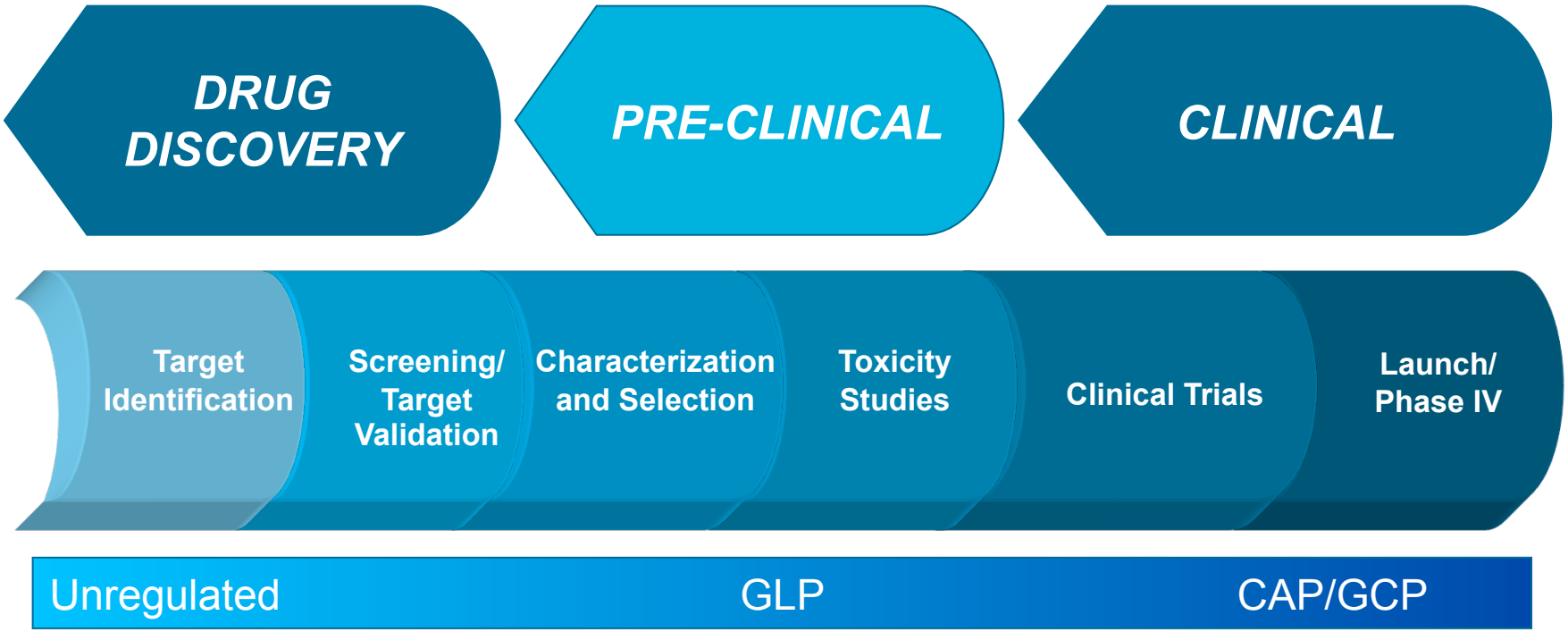
Validation Considerations

Assay Complexity

Surface phenotyping
- Simple/complex (IVD/RUO)
- Quantitative antigen expression (MESF/ABC)

- Intracellular
  - Cytokines
  - Nuclear proteins
  - Phosphorylated antigen detection (Phosflow)
- Neutralizing antibody (NAb), Anti-drug Antibody (ADA)
- Receptor Occupancy

Validation Considerations



- Stage of Drug Development
- Sample Type
- Methodology
- Data Type
- Intended Use

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Validation Considerations

2. Validation Stringency & Regulatory Requirements

- GLP, CLIA, GMP
- Establish what the method needs to do
  - Companion Diagnostic
  - Safety
  - Enrollment biomarker
  - PD biomarker
  - Exploratory biomarker


Lot release


Certificate of Analysis


Toxicity


Potency


PD Biomarker


Dosing

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Iterative Approach

Assay development

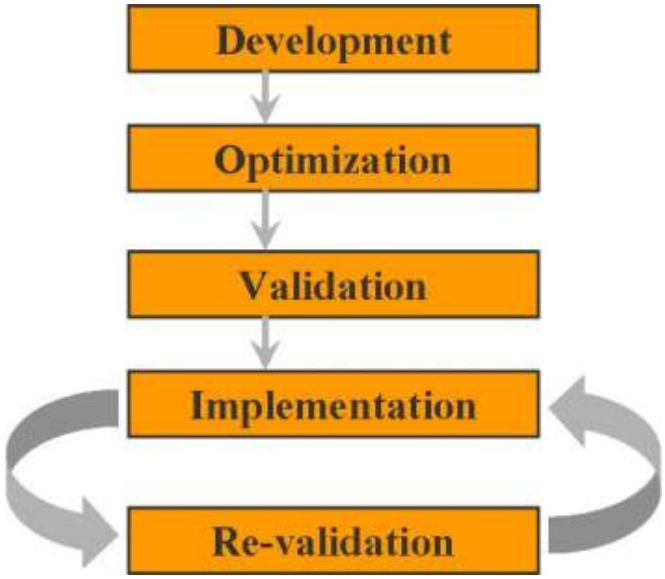
- ☑ State-of-the-art
- ☑ Fully optimized prior to validation

Assess performance

- ☑ Additional validation, as needed

Initial validation/record keeping

- ☑ Quality: Data must be able to be used if stringency requirements increase



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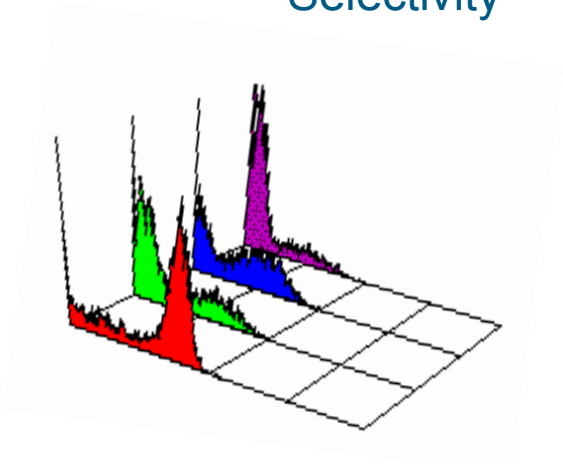
Validation of Flow Biomarker Methods

Validation Objective for Research-Use-Only (RUO), Lab Developed Test (LDT)

Establish method performance

- Accuracy
- Precision/Robustness
- Sensitivity/Limit of Detection
- Specificity
- Reference Intervals
- Stability
- Selectivity
- Standard Calibrators
- Range of Quantification
- Dilutional linearity
- Incurred Sample Reanalysis
- Interference (Matrix, Drug)
- Normal signal distribution
- Prozone effect



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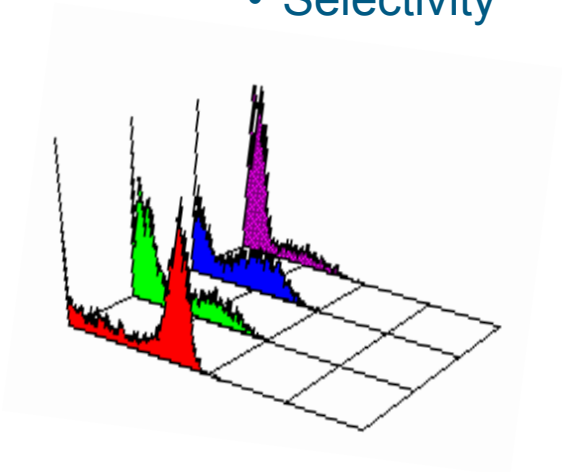

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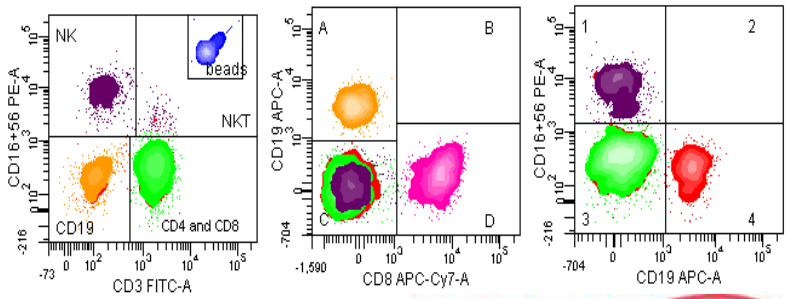
Specificity for Flow Biomarker Assays

NON-REG GLP CLIA

Immunophenotyping

- CD markers used to define the cellular population or antigens of interest must be justified from the literature
- Monoclonal antibody specificity should be verified by the Leucocyte Differentiation Antigens Workshops or peer reviewed publication
- Novel/custom mAb specificity must be well documented internally
- Gating strategies must be verified to establish the cell subset of interest is included while other cell subsets and non-specific events are excluded



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Accuracy

Standard Definition

- closeness of the result compared to the true value of the analyte

GLP

- Determined by the mean bias determined in spiked recovery experiments

CAP/CLIA

- comparison to “gold standard” method
  - measured concentrations in an official reference sample
  - measuring a concentration in comparison to an official standard

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Accuracy for Flow

Flow IVD

- CAP Proficiency Testing Surveys are available
- QC material with target values are available

Novel/Advanced Immunophenotyping

- Lack of proficiency testing programs
- Lack of cellular reference/QC material with target values for the populations of interest
- For novel or proprietary methods, sample exchange in not possible

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Specificity for Flow

CD markers used to define the cellular population or antigens of interest must be justified or recent published data sought

Monoclonal antibody specificity should be verified by the **Leucocyte Differentiation Antigens Workshops** or peer-reviewed publication

Gating strategies must be verified to establish:

- cell subset of interest is included
- other cell subsets and non-specific events are excluded

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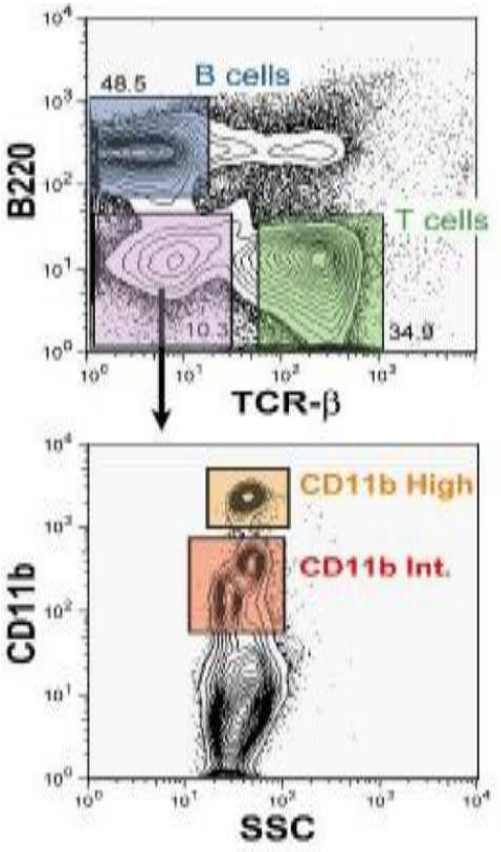
Precision for Flow

Precision

- Difficult to find samples with varying levels of each reportable result
  - Use different donors or QC material
- <10 %CV desirable for all methods
- <20-25 %CV acceptable for immunoassays per Fit-for-Purpose paper
- <30 %CV may be acceptable for rare event detection use as exploratory biomarkers
  - With poor precision, more replicates and samples are required

↓

"Iteration in validation"



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Sensitivity

Sensitivity

- the lowest reportable result

CAP/CLIA

- response above the limit of detection (LOD)

GLP

- lower limit of quantification (LLOQ) as the lowest concentration that can be measured with acceptable accuracy and precision (e.g., ± 20% CV)

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Sensitivity for Flow

Lower Limit of Detection (LOD)

- FMO controls +3 SD

Lower Limit of Quantitation (LLOQ)

- Difficult to find samples
- Mix stained and unstained samples
- Targeted cell depletion followed by re-spiking

Weighted importance for biomarker data

Need to know at what point are the results are imprecise

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Reference Intervals for Biomarkers

Reference Intervals

- Not required for first usage exploratory biomarkers
- Required for disease biomarkers or companion diagnostics (e.g. iterative approach)

Stability

- Acceptance criteria vs. precision

Costs

Not applicable for AML-MRD assessment in our case

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Validation Acceptance Criteria

CAP/CLIA

- criteria are established depending on the intended clinical use of an assay
- ATE

GLP

- Mean bias (% relative error) and % coefficient of variation should be less than 15% (20% at the LLOQ)

Fit for Purpose

- accuracy (mean bias) and imprecision (%CV) should be within 25% (30% for the LLOQ)
- Should meet the predefined needs of the study rather than simply reflecting the performance capabilities of the assay
- The nature of the assay methodology and the data generated using that assay
- The biological variability of the biomarker within and between the study populations

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Challenges of flow cytometry in global clinical trials

Advanced, High-Complexity Technology
Requires highly skilled staff

Limited Specimen Stability
Requires regional sample processing

Global Standardization
Necessary for site-to-site and longtitudinal data combinability

Lead Time for Assay Development and Validation

Validation Approach
Fit-for-purpose

Expense

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(Final) Validation Considerations

…that are critical in global clinical trials:

Reduce variability

- site-to-site
- longitudinally over time

Standardize

- instrumentation
- processes
- analysis (centralized gating)

Monitor performance

- via Standardization criteria
- QC variability (baseline for true response)

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Standardization, QC, and Biomarker Data in Clinical Trials

"The Goal in Quality Control is to ensure reduced instrument variation so biological variation can be measured."

From the Clinical Flow Cytometry in Hematological Malignancies Workshop, *"Standards and Controls in High-Dimensional Flow Cytometry"*, Stephen P. Perfetto, Staff Scientist and Director of the Flow Cytometry Core, NIAID, VRC

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AML-MRD assay

Potential Reportable Results (abridged)

Description of Potential Sub-classification and Phenotypes				
Population	**Phenotype**	**Reportable Result**	**Derived from Assay Tube**	**Comments**
M1-M2 Neutrophil lineage	$CD45^{low}$, CD34 (75%), CD33 (75%), CD117 (75%), CD13 (75%), HLA-DR (75%), CD15 (75%),	% of total $CD45^+$ leucocytes and a free text description of the phenotype	2	$HLA\text{-}DR^+$, CD4, and $CD14^-$
M3	$CD45^{low}$, CD34 (<10%), CD33 (>80%), CD117 (30%), CD13 (>80%), HLA-DR (<10%), CD15 (75%),	% of total $CD45^+$ leucocytes and a free text description of the phenotype	2	$HLA\text{-}DR^-$, CD4, and $CD14^-$
for erythroid lineage	$CD71^+$, $CD34^{-/+}$, $CD117^{-/+}$, $HLA\text{-}DR^{-/+}$, $CD13^+$	% of total $CD45^+$ leucocytes and a free text description of the phenotype	2	
M4	$CD45^{low}$, CD34 (<10%), CD117 (30-75%), HLA-DR (>80%), CD13(75%), CD64(>80%), $CD123^-$, CD4 or $CD14^+$	% of total $CD45^+$ leucocytes and a free text description of the phenotype	3	$CD13^+CD33^+$ MPO^-, CD4 or $CD14^+$ AML5 $CD13^+CD33^+MPO^+$ $HLA\text{-}DR^+$, CD4 or $CD14^+$
AML5	$CD45^{low}$, CD34 (<10%), CD117 (30-75%), HLA-DR (>80%), CD13(75%), CD64(>80%), $CD123^-$, CD4, or $CD14^+$	% of total $CD45^+$ leucocytes and a free text description of the phenotype	3	$CD13^+CD33^+$ MPO^-, CD4 or $CD14^+$ AML5 $CD13^+CD33^+MPO^+$ $HLA\text{-}DR^+$, CD4 or $CD14^+$

AML-MRD Assay

AML Configuration

- 5-tube, 8-color assay based on:

EuroFlow recommendations

[van Dongen et al., Leukemia (2012), 1908-1975.]

Work by Brent Woods and colleagues

[Wood et al., J Clin Oncol (2001), 29:1190-1197.]

Detector	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8
Fluor / Tube	BV421	BV510	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
FMO	HLA-DR	CD34		CD13				CD45
Exp1	HLA-DR	CD34	CD15	CD13	CD117	CD33	CD71	CD45
Exp2	HLA-DR	CD34	CD64	CD13	CD4	CD14	CD123	CD45
Exp3	HLA-DR	CD34	CD38	CD13	CD117	CD19	CD56	CD45
Exp4	HLA-DR	CD34	CD2	CD13	CD117	CD11b	CD7	CD45

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Acute myeloid leukemia (AML)

- clonal hematopoietic disorder
- derived from an HSC or lineage-specific progenitor cell
- ~20% blast population in bone marrow is expected at diagnosis (<20% for some genetic variants)
- Characterized by clonal heterogeneity, antigenic drift over course of disease
  - Phenotypic changes
  - Emergence of subclones
- Current standard for minimal residual disease (MRD) is 0.01% for AML and ALL.

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Acute myeloid leukemia (AML)

How do we define abnormalities?

- aberrant lineage expression (e.g. CD19, CD7, CD56) on myeloid blasts
- Blasts lacking lineage markers entirely
- Asynchrony of marker expression
  - early/progenitor markers (e.g. CD34, CD117) being co-expressed with more mature markers (e.g. CD33, CD13)
- Aberrant up- or down-modulation of Ag expression

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AML-MRD Assay

AML strategy for MRD detection

AML is clonally heterogeneous and can exhibit frequent antigenic changes

- take advantage of longtitudinality
    - use baseline to establish clonal phenotypes of interest
    - create individualized patient templates
    - monitor possible antigenic drift to ensure MRD detection is robust
    - follow multiple abnormal phenotypes to increase tracking specificity

Populations at MRD level can be lost in the noise of normal bone marrow

- Focus on blast population (“Les Bermudes”) which lacks mature cell types
- Look for “empty spaces” to migitate confounding effects of normal bone marrow populations
- Design a spiking technique to more closely mimic MRD during LLOQ experiments

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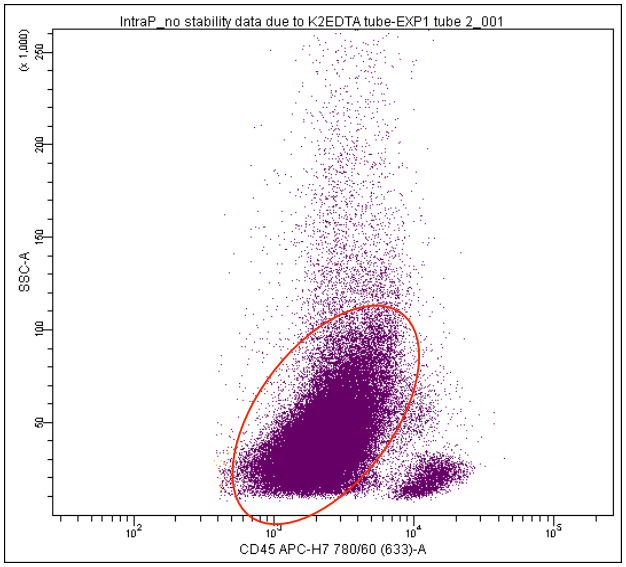
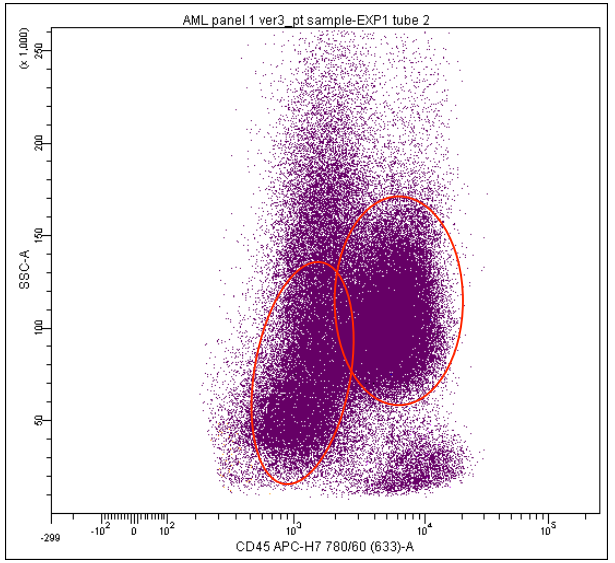
Variability in AML-MRD

Gross heterogeneity of AML samples



Defined blast population



Defined blast population
with extensive myelomonocytic expansion

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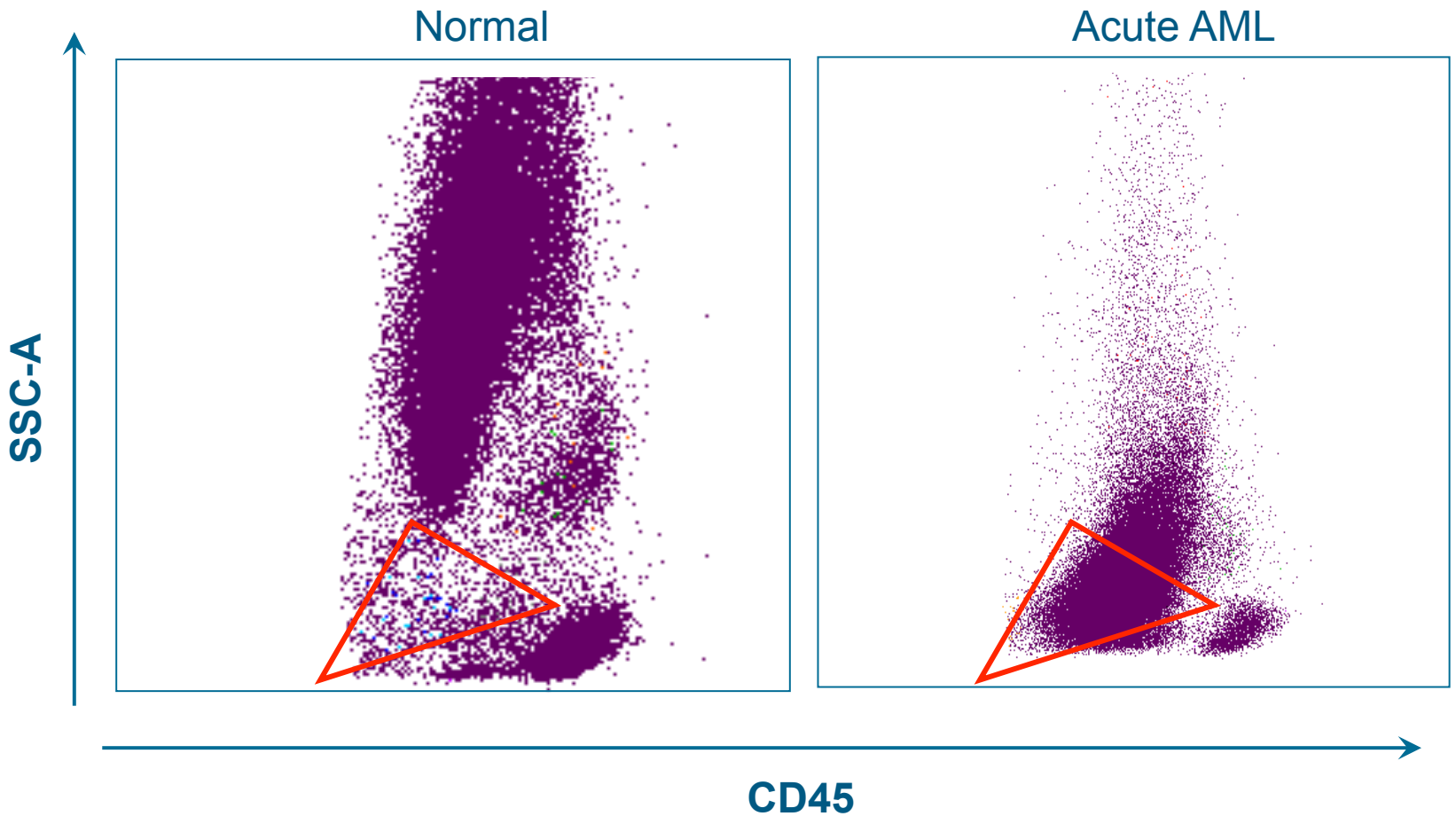
Normal vs. Acute Blasts in AML-MRD



red triangle; blast region/les “Bermudes”

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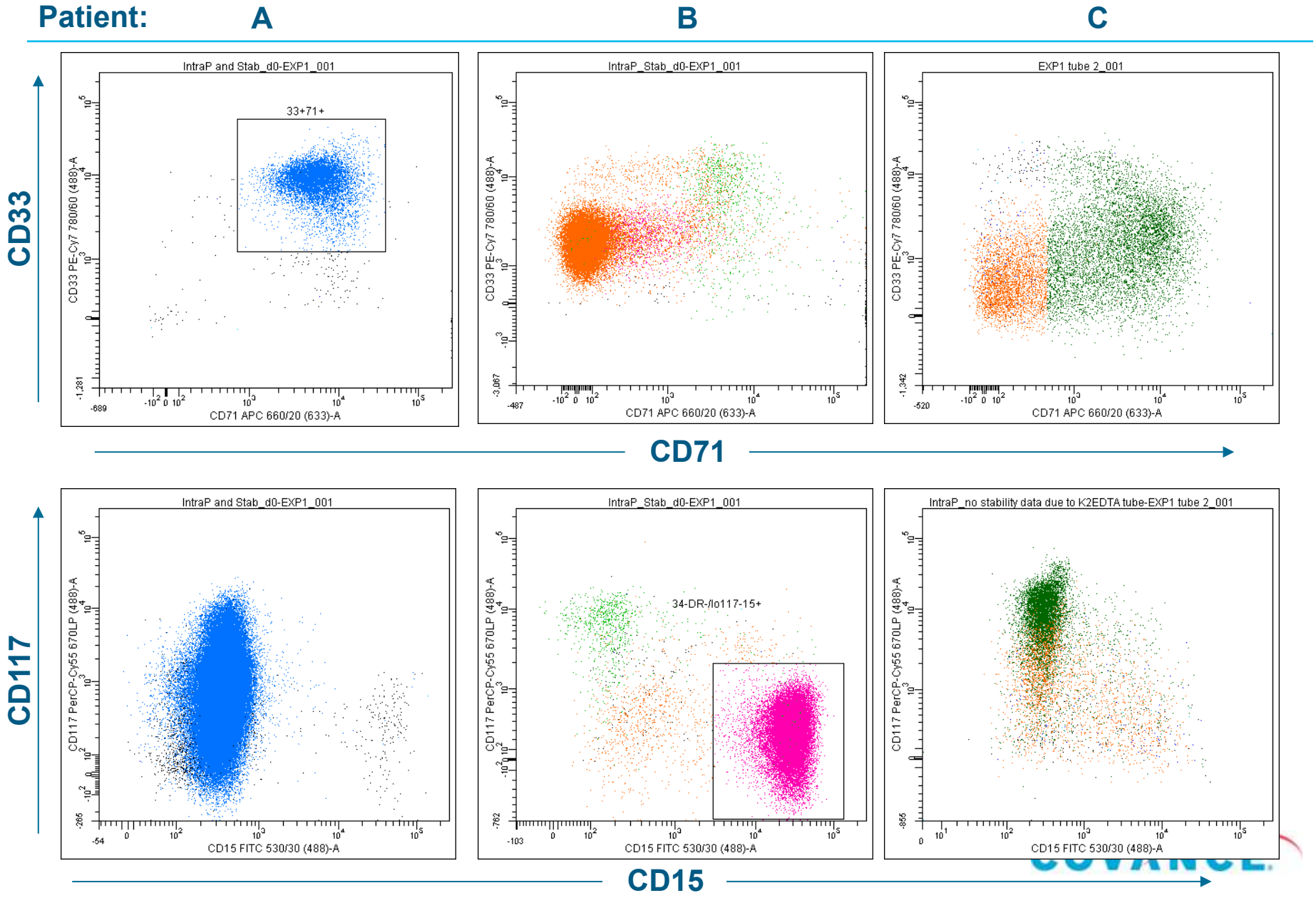
Immunophenotype heterogeneity in AML blasts



AML-MRD

Assay Performance Summary

Intra-assay imprecision (Intra-precison)

- 29 total phenotypically-distinct populations from 6 individual AML patients were assessed for (im)precision.
  - Imprecision ranged from 0.11% to 11.25%.
- 12 total phenotypically-distinct populations from 3 normal bone marrows were assessed for (im)precision.
  - Imprecision ranged from 0.71% to 8.81%.

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AML-MRD

Assay Performance Summary

Normal Bone Marrow

Table 6.4. Intra-assay Precision Summary – Normal Bone Marrow Samples

Relative Percentage (%)	Mean %CV*			Grand Mean %CV
	NBM1	NBM2	NBM3	
$CD2^+$	2.42	4.13	3.62	3.39
$CD4^+$, $CD123^+$	2.61	5.69	6.67	4.99
$CD7^+$	3.57	3.75	3.90	3.74
$CD11b^+$	1.46	0.07	0.61	0.71
$CD14^+$, $CD64^+$	2.17	0.32	2.04	1.51
$CD19^+$, $CD38^+$	5.89	4.79	2.54	4.41
$CD33^+$, $CD13^+$	1.67	0.98	2.87	1.84
$CD33^+$, $CD15^+$	5.89	1.44	0.78	2.70
$CD33^+$, $CD71^+$	1.55	1.95	2.78	2.09
$CD34^+$, $CD19^+$	16.90	4.76	4.76	8.81
$CD56^+$	1.65	3.96	2.08	2.56
$CD117^+$	4.50	4.51	3.28	4.10

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AML-MRD

Assay Performance Summary

LLOQ

- 8 total phenotypically-distinct populations from 3 individual patients were assessed for LLOQ
- 1 population was excluded due to extensive overlap with unspiked bone marrow (i.e. normal)
- LLOQ = 0.0027% (Assay limit set at LOD = 0.0037%)
- Equivalent to < 20 events in a background of 750,000-1,000,000 total events.

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AML-MRD

Assay Performance Summary

Table 6.1. Analytical Sensitivity / LOD

Relative Percentage (%)	Un-spiked Rep1	Un-spiked Rep 2	Un-spiked Rep 3	Mean	SD	%CV	Mean +3SD
$CD13^{lo}$, $CD15^{+}$, $CD117^{-}$	0.01150	0.01782	0.01957	0.0163	0.0042	25.767	0.02890
$CD13^{-}$, $CD15^{+}$	0.00068	0.00000	0.00059	0.0004	0.00037	92.500	0.00151
$CD13^{hi}$, $CD64^{-/lo}$	0.00086	0.00084	0.00088	0.0009	0.0000	2.3256	0.0009
$CD34^{-}$, HLA-DRhi, $CD13^{hi}$, $CD11b^{+}$	0.00078	0.00065	0.00058	0.0007	0.0001	14.9254	0.0010
$CD34^{+}$, $CD13^{+}$	0.00260	0.00229	0.00170	0.0022	0.0005	22.7273	0.0037
$CD34^{hi}$, $CD123^{+}$	0.00140	0.00093	0.00121	0.0012	0.0002	16.6667	0.0018
$CD34^{+}$, $CD13^{hi}$, $CD38^{dim}$	0.00104	0.00066	0.00086	0.0009	0.0002	22.2222	0.0015
$CD34^{+}$, $CD13^{hi}$, $CD7^{+}$	0.00008	0.00014	0.00015	0.00012	0.00004	33.333	0.00024
Number of Events	**Un-spiked Rep1**	**Un-spiked Rep 2**	**Un-spiked Rep 3**	**Mean**	**SD**	**%CV**	**Mean +3SD**
$CD13^{lo}$, $CD15^{+}$, $CD117^{-}$	17	24	33	24.667	8.021	32.517	48.730
$CD13^{-}$, $CD15^{+}$	1	0	1	0.6667	0.577	86.546	2.398
$CD13^{hi}$, $CD64^{-/lo}$	9	8	9	8.6700	0.5770	6.6551	10.4010
$CD34^{-}$, HLA-DRhi, $CD13^{hi}$, $CD11b^{+}$	8	7	6	7.0000	1.0000	14.2857	10.0000
$CD34^{+}$, $CD13^{+}$	26	23	17	22.0000	4.5830	20.8318	35.7490
$CD34^{hi}$, $CD123^{+}$	15	10	13	12.6700	2.5170	19.8658	20.2210
$CD34^{+}$, $CD13^{hi}$, $CD38^{dim}$	11	7	9	9.0000	2.0000	22.2222	15.0000
$CD34^{+}$, $CD13^{hi}$, $CD7^{+}$	1	2	2	1.67	0.577	34.551	3.401

AML-MRD

Assay Performance Summary

Table 6.2.e. $CD13^{hi}$,$CD64^{-/lo}$ LLOQ2 - Relative Percentage (%)

Dilution level	LLOQ2					
	Rep1	Rep2	Rep3	Mean	SD	%CV
1	0.00059	0.00054	0.00024	0.00046	0.00019	41.30
2	0.00031	0.00060	0.00108	0.00066	0.00039	59.09
3	0.00323	0.00224	0.00268	0.0027	0.0005	18.52

Table 6.2.f. $CD13^{hi}$,$CD64^{-/lo}$ LLOQ2 - Events

Dilution Factor	LLOQ2					
	Rep1	Rep2	Rep3	Mean	SD	%CV
1	5	5	2	4.000	1.732	43.30
2	2	4	7	4.333	2.517	58.09
3	23	16	19	19.333	3.512	18.17

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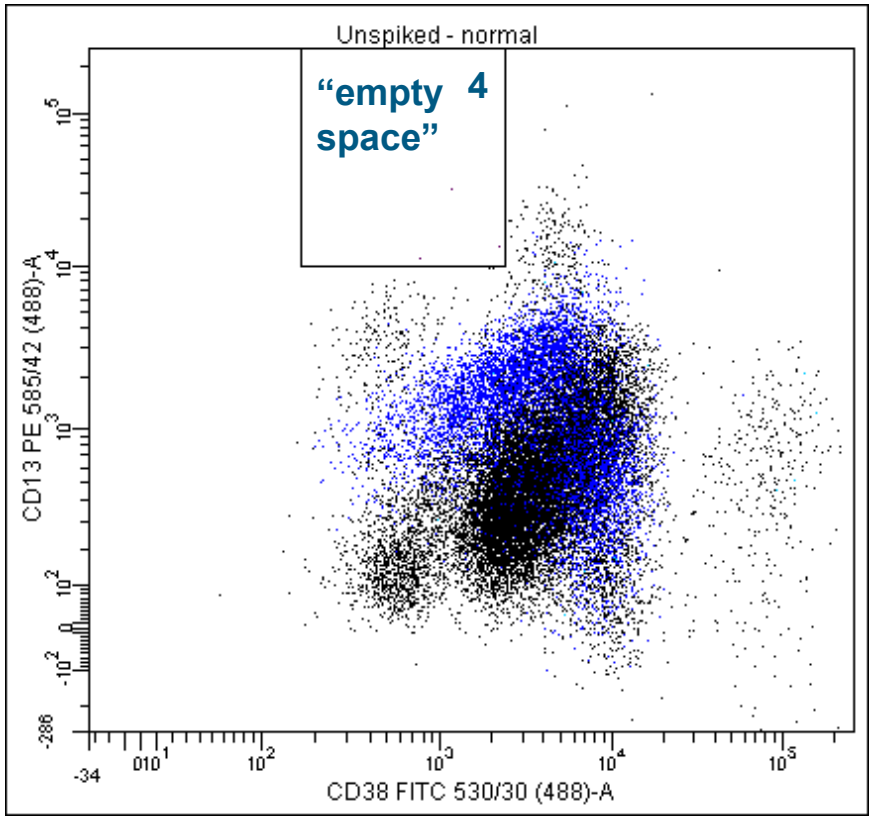
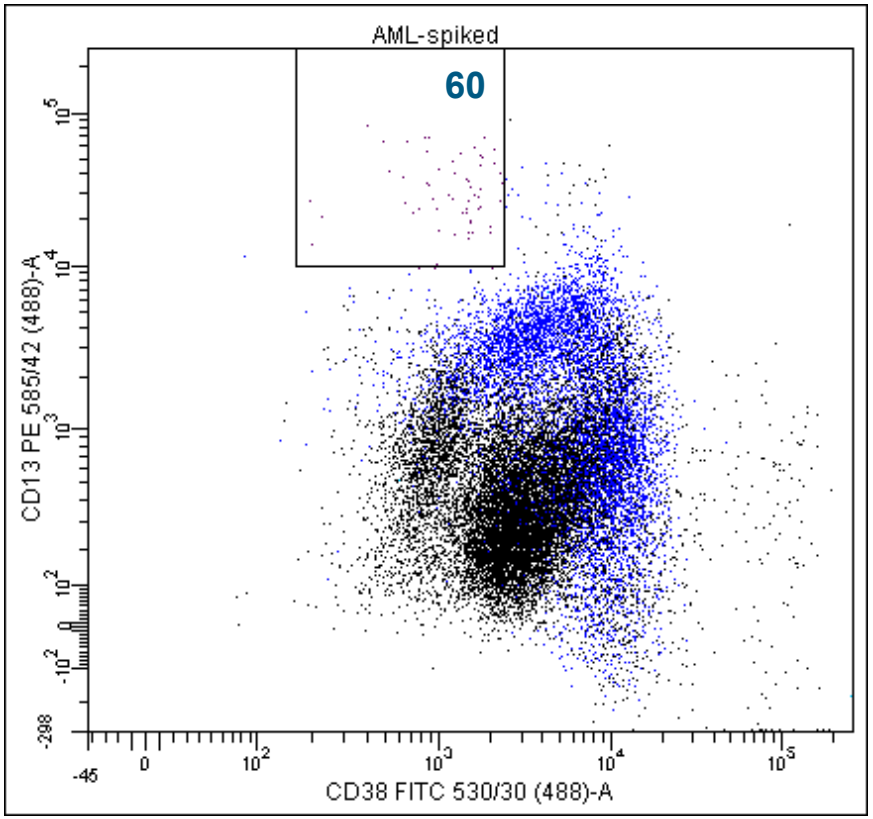
Detection of AML cells in normal bone marrow

Normal, unspiked



AML-spiked



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AML-MRD

Assay Performance Summary

QC material

Table 6.5. Inter-assay Precision Summary

Relative Percentage (%)	%CV		Grand Mean %CV
	CDSS1	CDSS2	
$CD2^+$	8.56	13.21	10.89
$CD4^+$	13.31	15.21	14.26
$CD7^+$	12.52	12.52	12.52
$CD11b^+$, $CD13^+$	1.41	5.64	3.53
$CD13^+$, $CD33^+$	11.36	9.58	10.47
$CD14^+$, $CD64^+$	7.64	2.71	5.18
$CD15^+$	1.07	3.07	2.07
$C19^+$	0.93	1.12	1.03
$CD38^+$	10.88	11.73	11.31
$CD56^+$	13.51	16.02	14.77
$CD71^+$, $CD117^+$	16.74	8.23	12.49
$CD123^+$	3.95	7.84	5.90

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AML-MRD

Assay Performance Summary

Table 6.8. Specimen Stability Summary – Abnormal Bone Marrow

Relative Percentage (%)	Day 1		Day 2	
	% Change	%CV	% Change	%CV
ABM1	23.60	21.77	16.91	13.94
ABM2	9.82	6.83	9.24	6.94
ABM3	18.67	11.78	45.82	24.72
ABM6	16.43	13.07	23.71	19.44

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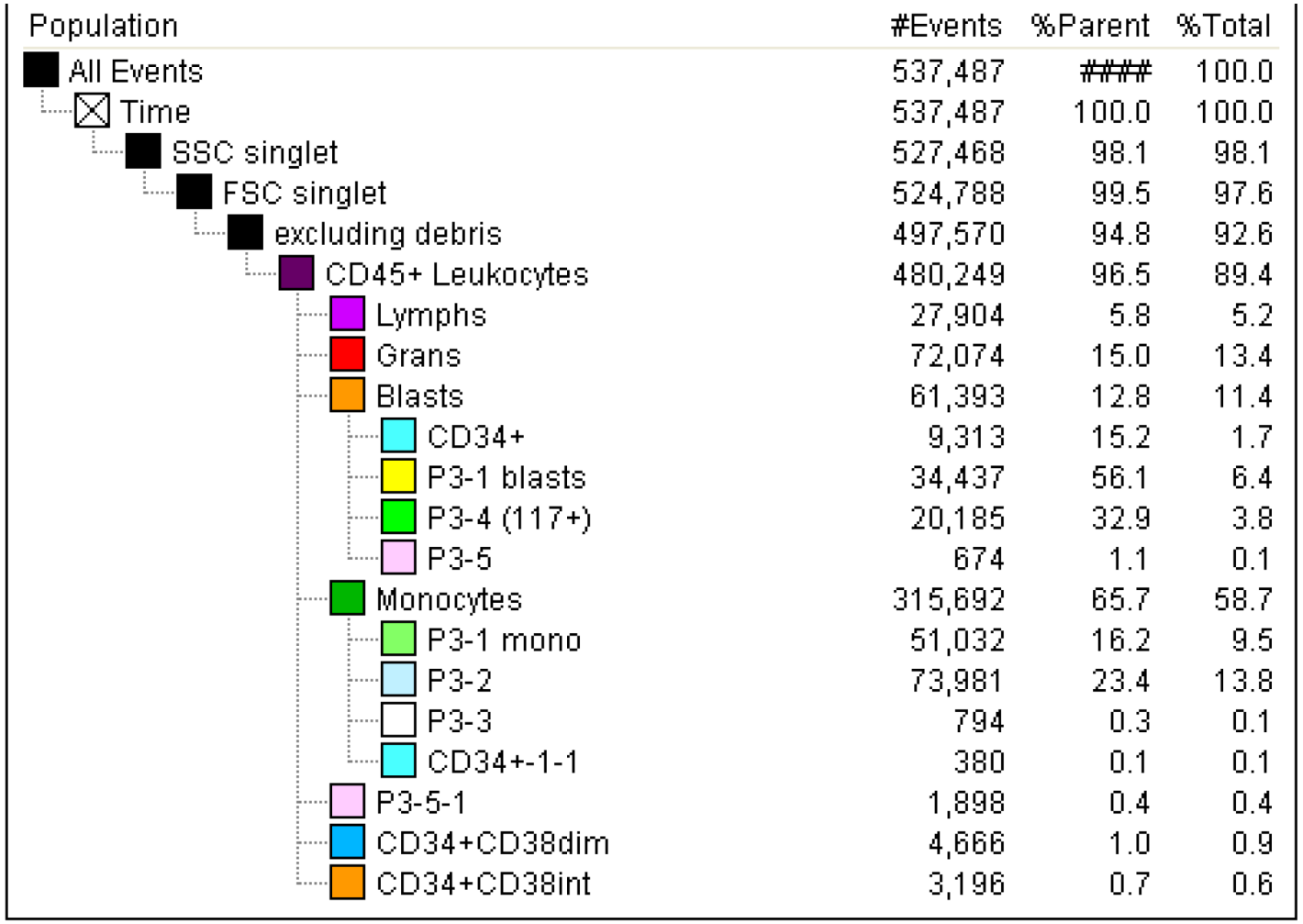
AML-MRD as a Flow Biomarker Assay



Population	#Events	%Parent	%Total
All Events	537,487	####	100.0
Time	537,487	100.0	100.0
SSC singlet	527,468	98.1	98.1
FSC singlet	524,788	99.5	97.6
excluding debris	497,570	94.8	92.6
CD45+ Leukocytes	480,249	96.5	89.4
Lymphs	27,904	5.8	5.2
Grans	72,074	15.0	13.4
Blasts	61,393	12.8	11.4
CD34+	9,313	15.2	1.7
P3-1 blasts	34,437	56.1	6.4
P3-4 (117+)	20,185	32.9	3.8
P3-5	674	1.1	0.1
Monocytes	315,692	65.7	58.7
P3-1 mono	51,032	16.2	9.5
P3-2	73,981	23.4	13.8
P3-3	794	0.3	0.1
CD34+-1-1	380	0.1	0.1
P3-5-1	1,898	0.4	0.4
CD34+CD38dim	4,666	1.0	0.9
CD34+CD38int	3,196	0.7	0.6

AML-MRD as a Flow Biomarker Assay

Reportable Results	
Population	Phenotype
% MRD	**Patient-specific**
Phenotype	**Free text (consulting hematopathologist)**

Acknowledgments

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Questions?

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