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Current Landscape in Minimal Residual Disease (MRD) Testing in Hematologic Malignancies

USA-OCF-034182(1)

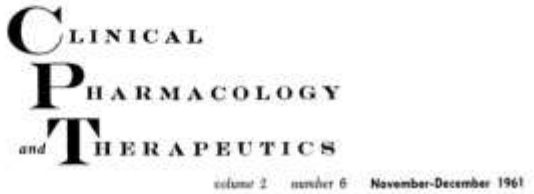
Outline

- Response evaluation in hematologic malignancies
- Technical aspects of MRD measurement
  - Flow cytometry
  - Quantitative PCR
  - Next generation sequencing
- Incorporation of MRD results into patient management
  - Patient monitoring
  - Risk stratification and treatment decisions
  - Trial endpoints
- Summary

Response Assessments: The Basics

> "With the enormous increase in the clinical use of drugs to control the growth of cancer, it becomes essential to develop an **acceptable** and **meaningful terminology** to describe the indications and therapeutic effectiveness of each agent or procedure. The orderly presentation of information would **serve as a guide to the practicing physician** and it would provide a common language to facilitate the collection and analysis of clinical experiences."

David A. Karnofsky



- Response Assessments:
  - Provide prognostic information
  - Simplify the evaluation of new agents

Karnofsky DA. *Clin Pharmacol Ther.* 1961;2:709-712.

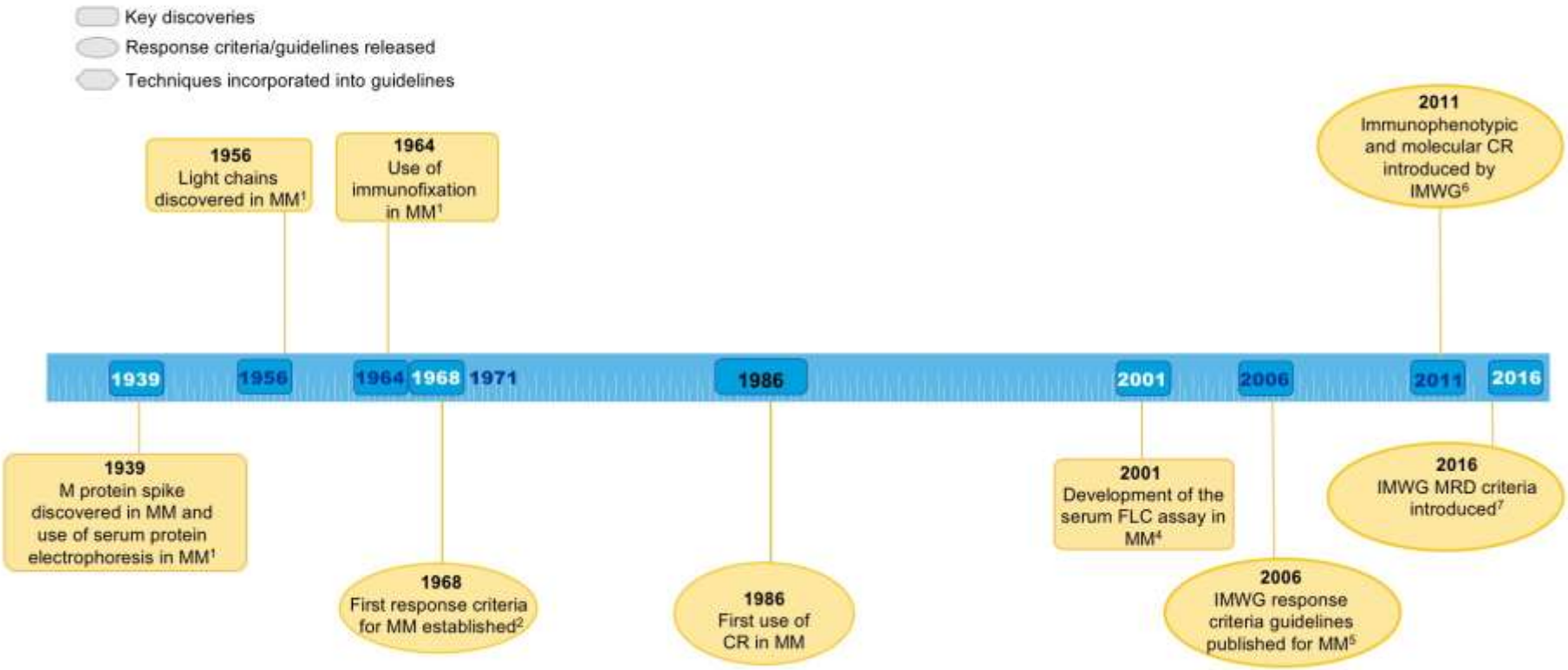
Established Techniques for Response Assessment

	Microscopy/Morphology	PET/CT or Radiology	Flow Cytometry	Serology
ALL[1,2]	✓	±		
AML[2,3]	✓	±		
CLL[4]	✓	✓	✓	
CML[5]	✓			
NHL[3,6]	✓	✓	✓	±
MM[7-9]	✓	✓	✓	✓

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; IHC, immunohistochemistry; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; PET-CT, positron emission tomography- computed tomography.

1. Applebaum FR, et al. *Blood*. 2007;109:1810-1816; 2. Cunningham I, Kohno B. *Am J Hematol*. 2016;91:379-384; 3. Cheson BD, et al. *J Clin Oncol*. 2007;21:4642-4649; 4. Hallek M, et al. *Blood*. 2008;111:5446-5456; 5. Jabbour E, et al. *Blood*. 2011;117:3641-3647; 6. Cheson BD, et al. *J Clin Oncol*. 2014;32:3059-3067; 7. Mailankody S, et al. *Nat Rev Clin Oncol*. 2015;12:286-295. 8. Mihailovic J, et al. *Semin Nucl Med*. 2015;45:16-31; 9. Kumar S, et al. *Lancet Oncol*. 2016;17:e328-e346.

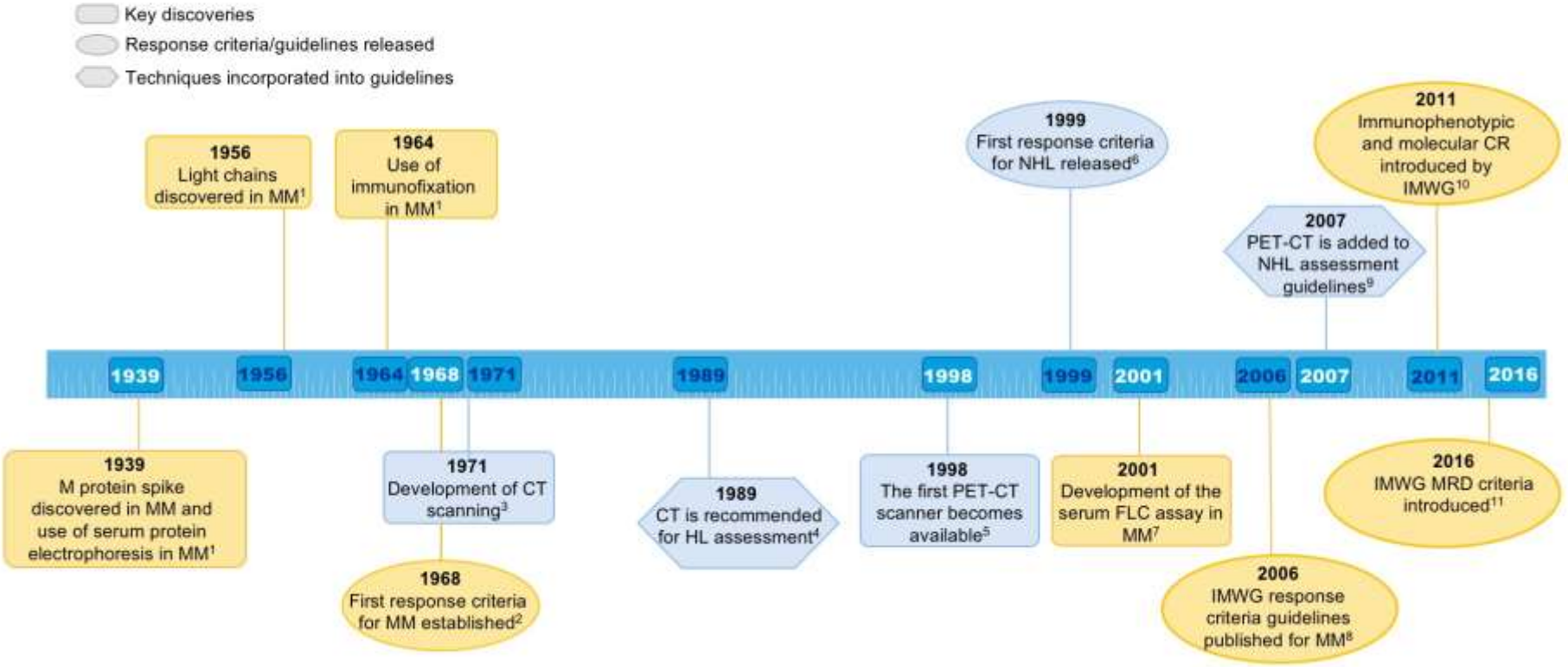
Timeline of Selected Response Criteria in Hematologic Malignancies – Multiple Myeloma



FLC, free light chain; IMWG, International Myeloma Working Group

1. Kyle RA, et al. *Blood*. 2008;111:2962-2972; 2. Blade J, et al. *Br J Haematol*. 1998;102:1115-1123; 3. Petrik V, et al. *Neurosurgery*. 2006;58:780-787; 4. Bradwell AR, et al. *Clin Chem*. 2001;47:673-680; 5. Durie BG, et al. *Leukemia*. 2006;20:1467-1473; 6. Rajkumar SV, et al. *Blood*. 2011;117(18):4691-4695; 7. Kumar S, et al. *Lancet Oncol*. 2016;17:e328-e346.

Timeline of Selected Response Criteria in Hematologic Malignancies – Lymphoma



HL, Hodgkin lymphoma.

1. Kyle RA, et al. *Blood*. 2008;111:2962-2972; 2. Blade J, et al. *Br J Haematol*. 1998;102:1115-1123; 3. Petrik V, et al. *Neurosurgery*. 2006;58:780-787; 4. Lister TA, et al. *J Clin Oncol*. 1989;7:1630-1636; 5. Townsend DW. *Semin Ultrasound CT MR*. 2008;29:232-235; 6. Cheson BD, et al. *J Clin Oncol*. 2014;32:3059-3067; 7. Bradwell AR, et al. *Clin Chem*. 2001;47:673-680; 8. Durie BG, et al. *Leukemia*. 2006;20:1467-1473; 9. Cheson BD, et al. *J Clin Oncol*. 2007;25:579-586; 10. Rajkumar SV, et al. *Blood*. 2011;117(18):4691-4695; 11. Kumar S, et al. *Lancet Oncol*. 2016;17:e328-e346.

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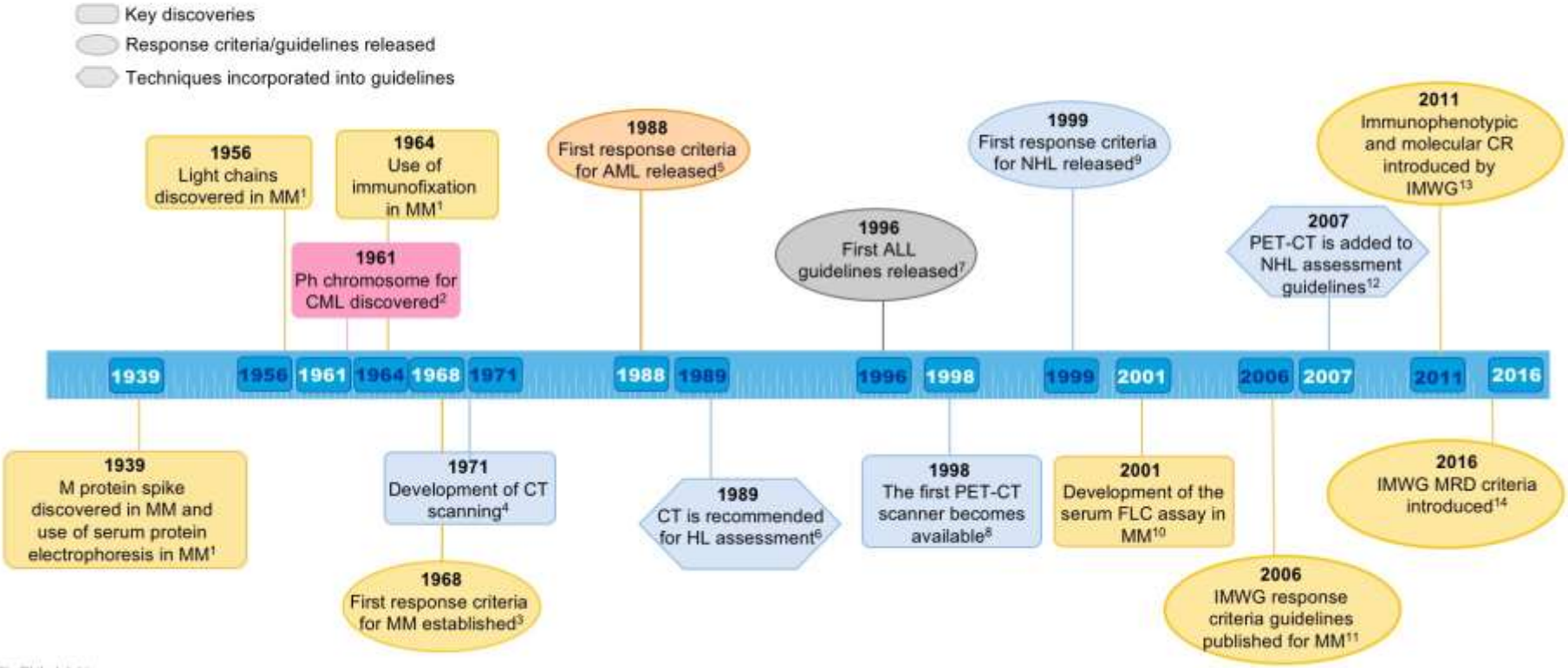
Timeline of Selected Response Criteria in Hematologic Malignancies – Leukemia



Ph, Philadelphia.

1. Kyle RA, et al. *Blood*. 2008;111:2962-2972; 2. Nowell PC, et al. *J Natl Cancer Instit*. 1961;27:1013-1035; 3. Blade J, et al. *Br J Haematol*. 1998;102:1115-1123; 4. Petrik V, et al. *Neurosurgery*. 2006;58:780-787; 5. Cheson BD, et al. *J Clin Oncol*. 2003;21:4642-4649; 6. Lister TA, et al. *J Clin Oncol*. 1989;7:1630-1636; 7. NCCN History. National Comprehensive Cancer Network website. www.nccn.org/about/history.asp. Accessed November 15, 2016; 8. Townsend DW. *Semin Ultrasound CT MR*. 2008;29:232-235; 9. Cheson BD, et al. *J Clin Oncol*. 2014;32:3059-3067; 10. Bradwell AR, et al. *Clin Chem*. 2001;47:673-680; 11. Durie BG, et al. *Leukemia*. 2006;20:1467-1473; 12. Cheson BD, et al. *J Clin Oncol*. 2007;25:579-586; 13. Rajkumar SV, et al. *Blood*. 2011;117(18):4691-4695; 14. Kumar S, et al. *Lancet Oncol*. 2016;17:e328-e346.

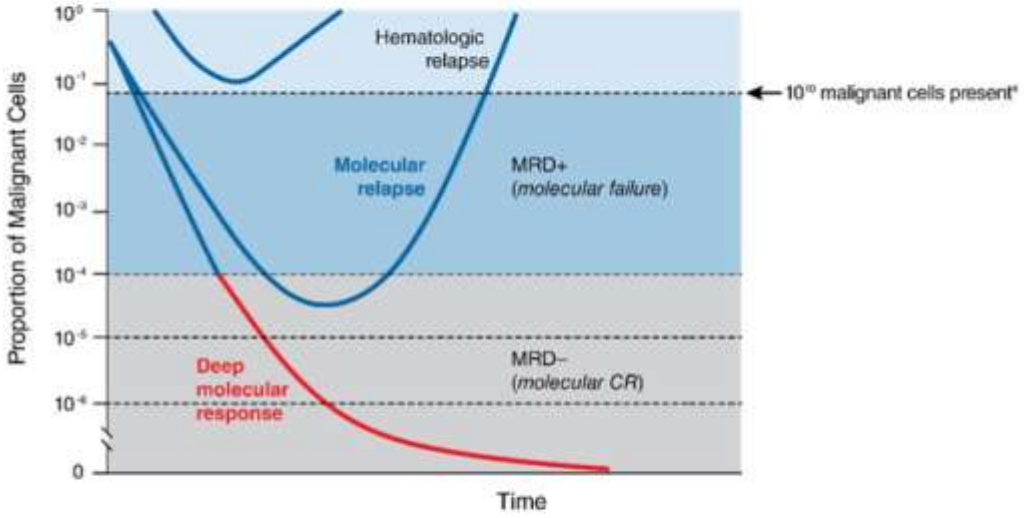
Complete Response

- Complete response or remission is often accepted as a predictor of clinical benefit
- Limitations of complete response
  - Presence of sanctuary sites[1]
  - Sampling errors[2]
  - Insensitive assays[3,4]

1. Inaba H, et al. *Lancet*. 2013;381:1943-1955; 2. Mallankody S, et al. *Nat Rev Clin Oncol*. 2015;12:286-295; 3. Ommen HB, et al. *Blood*. 2010:198-205; 4. Cortes J, et al. *Cancer*. 2011;117:1113-1122.

Minimal/Measurable Residual Disease (MRD)

- MRD is the presence of malignant cells below the detection limit of conventional methods[1,2]
- Routine MRD assessments have become a key element in the management of patients with hematologic malignancies[2,3]



Graphical definitions of MRD.
Adapted from Bruggemann M, et al.[2]

1. Inaba H, et al. *Lancet*. 2013;381:1943-1955; 2. Bruggemann M, et al. *Blood*. 2012;120:4470-4481; 3. Paiva B, et al. *Blood*. 2008;12:4017-4023; 4. Hauwel M, et al. *Swiss Med Wkly*. 2014;144:w13907.

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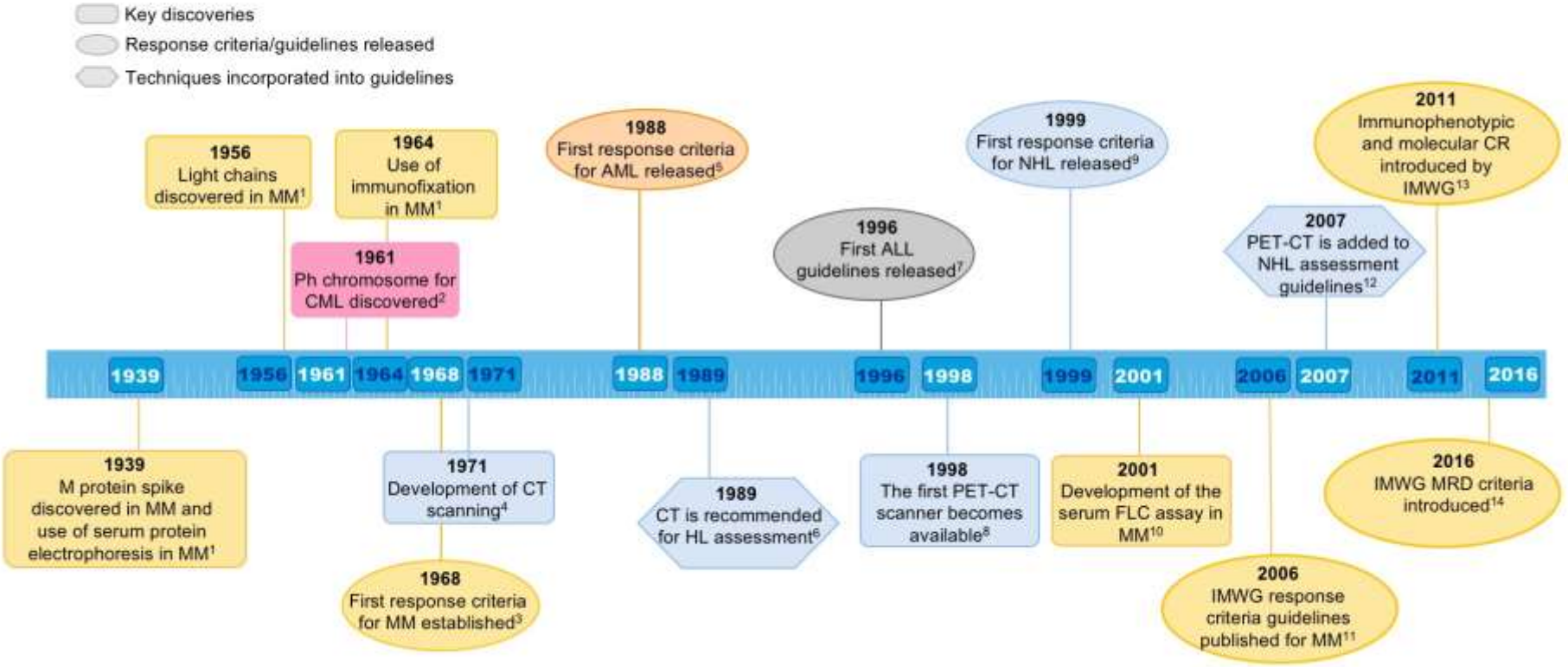
Measuring MRD

Timeline of Selected Response Criteria in Hematologic Malignancies



1. Kyle RA, et al. *Blood*. 2008;111:2962-2972; 2. Nowell PC, et al. *J Natl Cancer Instit*. 1961;27:1013-1035; 3. Blade J, et al. *Br J Haematol*. 1998;102:1115-1123; 4. Petrik V, et al. *Neurosurgery*. 2006;58:780-787; 5. Cheson BD, et al. *J Clin Oncol*. 2003;21:4642-4649; 6. Lister TA, et al. *J Clin Oncol*. 1989;7:1630-1636; 7. NCCN History. National Comprehensive Cancer Network website. www.nccn.org/about/history.asp. Accessed November 15, 2016; 8. Townsend DW. *Semin Ultrasound CT MR*. 2008;29:232-235; 9. Cheson BD, et al. *J Clin Oncol*. 2014;32:3059-3067; 10. Bradwell AR, et al. *Clin Chem*. 2001;47:673-680; 11. Durie BG, et al. *Leukemia*. 2006;20:1467-1473; 12. Cheson BD, et al. *J Clin Oncol*. 2007;25:579-586; 13. Rajkumar SV, et al. *Blood*. 2011;117(18):4691-4695; 14. Kumar S, et al. *Lancet Oncol*. 2016;17:e328-e346.

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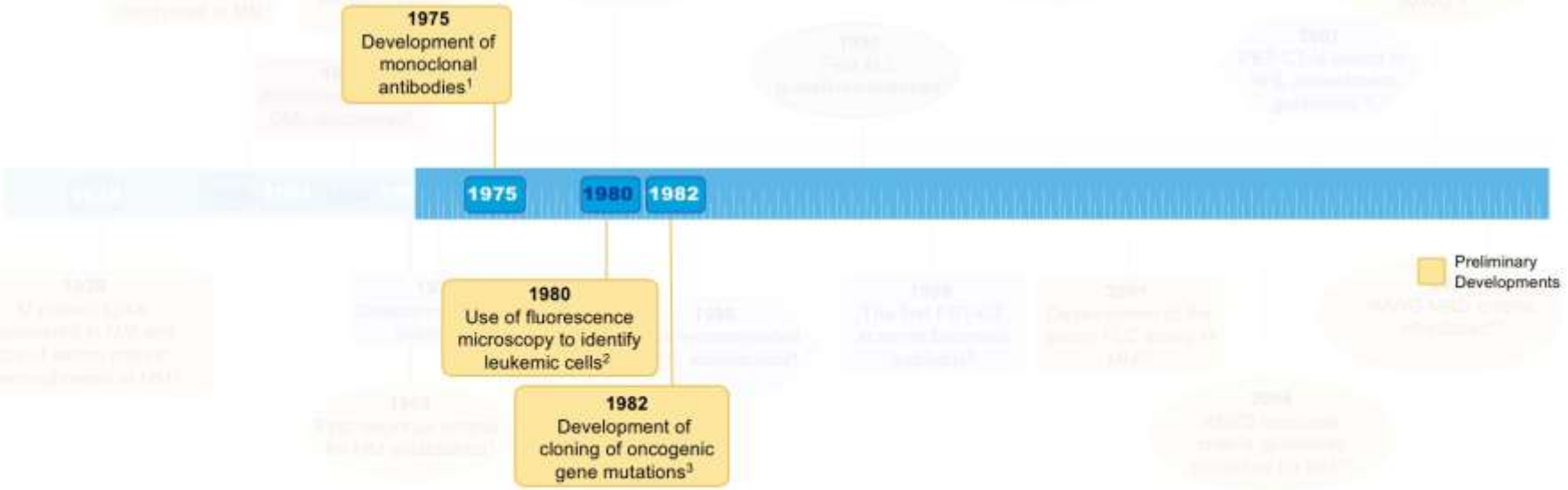
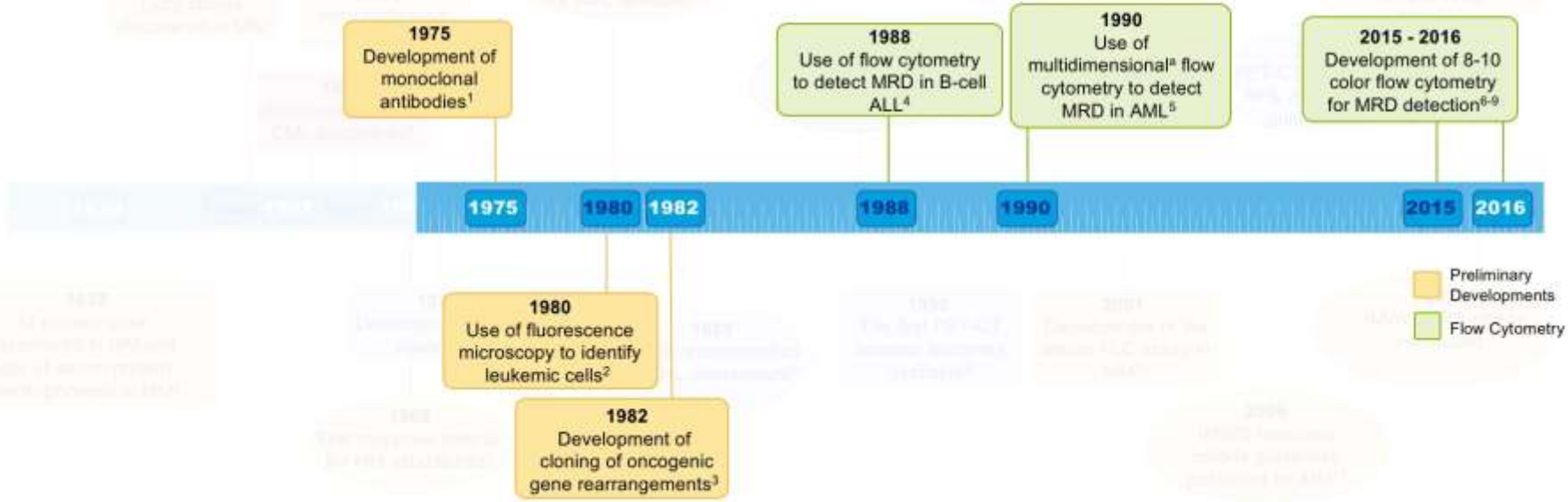
Selected Timeline of MRD Testing Development



1. Kohler G, et al. *Nature*. 1975;256:495-497; 2. Janossy G, et al. *Blood*. 1980;56:430-441; 3. Adams JM, et al. *Proc Natl Acad Sci USA*. 1982;79:6966-6970.

Selected Timeline of MRD Testing Development



[a] 2 light scattering channels and 3 fluorescence markers.

1. Kohler G, et al. *Nature*. 1975;256:495-497; 2. Janossy G, et al. *Blood*. 1980;56:430-441; 3. Adams JM, et al. *Proc Natl Acad Sci USA*. 1982;79:6966-6970; 4. Hurwitz CA, et al. *Blood*. 1988;72:299-307; 5. Terstappen LW, et al. *Analytical Cell Pathol*. 1990;2:229-240; 6. Karawajew L, et al. *Haematologica*. 2015;100:938-944; 7. Dowling AK, et al. *Lab Med*. 2016;47:103-111; 8. Zhou Y, et al. *Leukemia*. 2016;30:1456-1464; 9. Cheminant M, et al. *Haematologica*. 2016;101:336-345.

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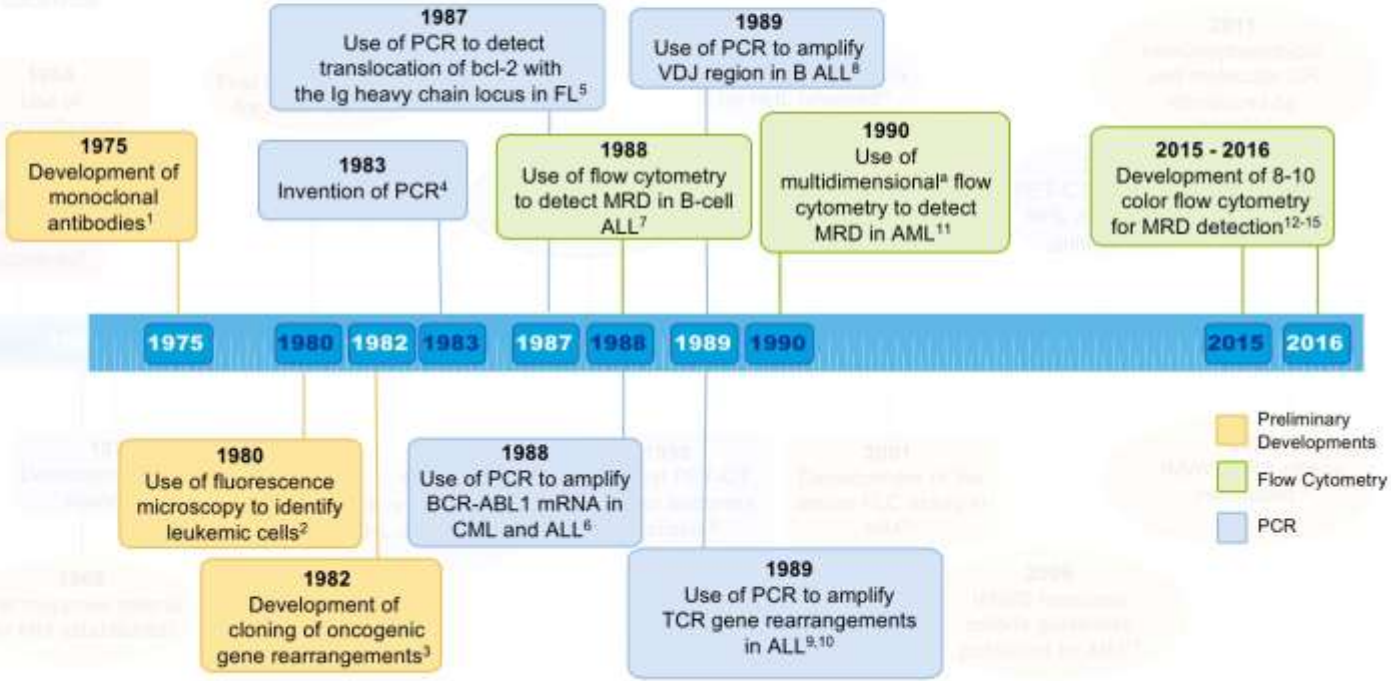
Selected Timeline of MRD Testing Development



[a]2 light scattering channels and 3 fluorescence markers.

1. Kohler G, et al. *Nature*. 1975;256:495-497; 2. Janossy G, et al. *Blood*. 1980;56:430-441; 3. Adams JM, et al. *Proc Natl Acad Sci USA*. 1982;79:6966-6970; 4. History of PCR. https://molecular.roche.com/innovation/pcr/history. Accessed November 22,2016; 5. Lee MS, et al. *Science*. 1987;237:175-178; 6. Kawasaki ES, et al. *Proc Natl Acad Sci USA*. 1988;85:5698-5702; 7. Hurwitz CA, et al. *Blood*. 1988;72:299-307; 8. Yamada M, et al. *Proc Natl Acad Sci USA*. 1989;86:5123-5127; 9. Hansen-Hagge TE, et al. *Blood*. 1989;74:1762-1767; 10. d'Auriol L, et al. *Leukemia*. 1989;3:155-158; 11. Terstappen LW, et al. *Analytical Cell Pathol*. 1990;2:229-240; 12. Karawajew L, et al. *Haematologica*. 2015;100:935-944; 13. Dowling AK, et al. *Lab Med*. 2016;47:103-111; 14. Zhou Y, et al. *Leukemia*. 2016;30:1456-1464; 15. Cheminant M, et al. *Haematologica*. 2016;101:336-345.

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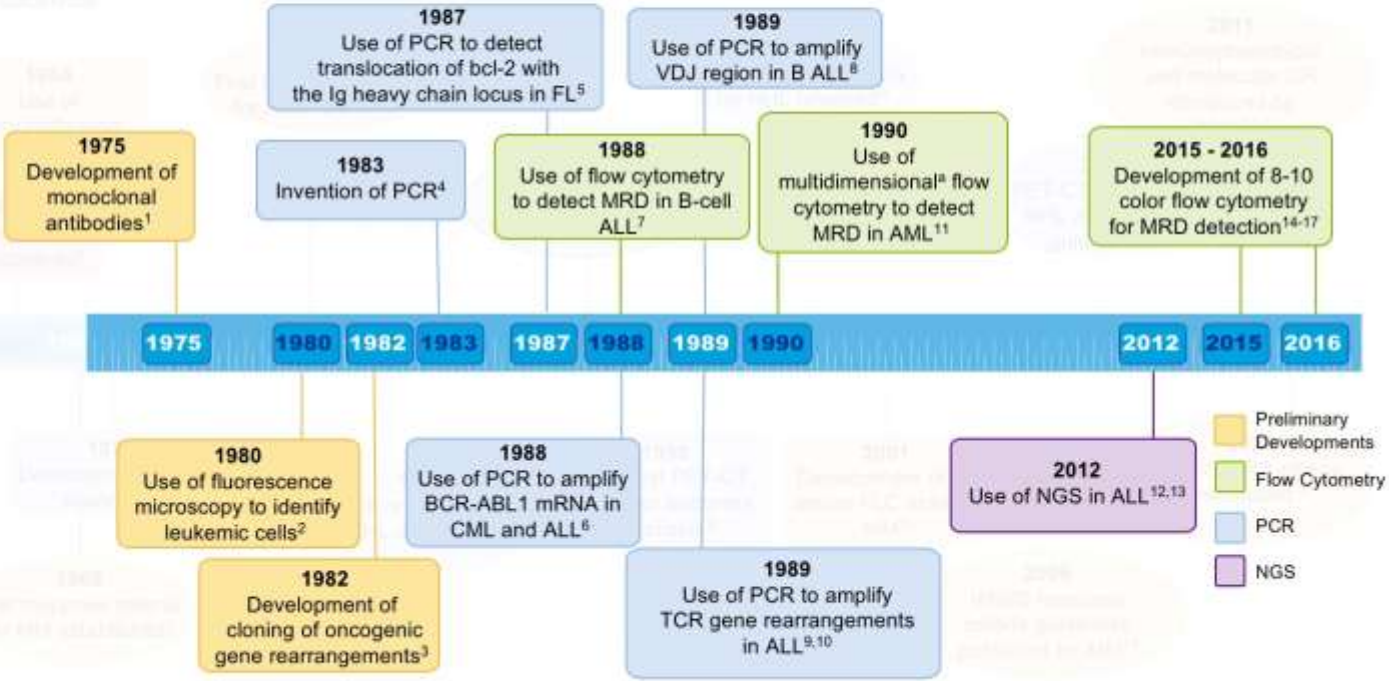

[a]2 light scattering channels and 3 fluorescence markers.
NGS, next generation sequencing.

1. Kohler G, et al. *Nature*. 1975;256:495-497; 2. Janossy G, et al. *Blood*. 1980;56:430-441; 3. Adams JM, et al. *Proc Natl Acad Sci USA*. 1982;79:6966-6970; 4. History of PCR. https://molecular.roche.com/innovation/pcr/history. Accessed November 22,2016; 5. Lee MS, et al. *Science*. 1987;237:175-178; 6. Kawasaki ES, et al. *Proc Natl Acad Sci USA*. 1988;85:5698-5702; 7. Hurwitz CA, et al. *Blood*. 1988;72:299-307; 8. Yamada M, et al. *Proc Natl Acad Sci USA*. 1989;86:5123-5127; 9. Hansen-Hagge TE, et al. *Blood*. 1989;74:1762-1767; 10. d'Auriol L, et al. *Leukemia*. 1989;3:155-158; 11. Terstappen LW, et al. *Analytical Cell Pathol*. 1990;2:229-240; 12. Faham M, et al. *Blood*. 2012;120:5173-5180; 13. Wu D, et al. *Sci Transl Med*. 2012;4:134ra63; 14. Karawajew L, et al. *Haematologica*. 2015;100;935-944; 15. Dowling AK, et al. *Lab Med*. 2016;47:103-111; 16. Zhou Y, et al. *Leukemia*. 2016;30:1456-1464; 17. Cheminant M, et al. *Haematologica*. 2016;101:336-345.

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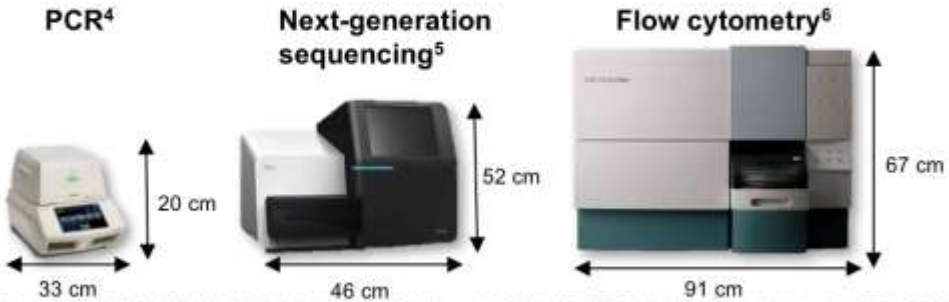
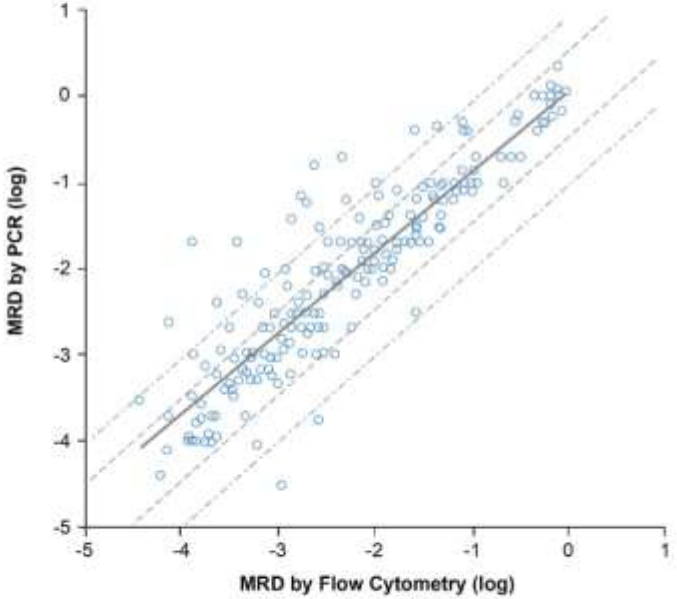
Techniques for Measuring MRD

- There are currently 2 established methods in use for determining MRD[1]
  - Flow Cytometry: sensitivity of 10^{-3} – 10^{-4}
  - PCR: sensitivity of 10^{-4} – 10^{-5}
- Paired analysis shows high levels of concordance between the two methods[2,3]

Approximate Equipment Sizes





Adapted from Denys B, et al[7]

1. Bruggemann M, et al. *Blood*. 2012;120:4470-4481; 2. Kerst G, et al. *Br J Haematol*. 2005;128:774-782; 3. Neale GA, et al. *Leukemia*. 2004;18:934-938; 4. Thermo Cycler Spec Sheet. http://www.bio-rad.com/en-us/product/c1000-touch-thermal-cycler?pcp_loc=catprod.Accessed 11/21/16; 5. Sequencing Spec Sheet. http://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/miniseq-system-specification-sheet-770-2015-039.pdf. Accessed 11/21/16; 6. Flow Cytometer Spec Sheet. http://www.bdbiosciences.com/documents/FACSCalibur_FlowCytometry_TechSpec.pdf. Accessed 11/21/16. 7. Denys B, et al. *Leukemia*. 2013;27:635-641.

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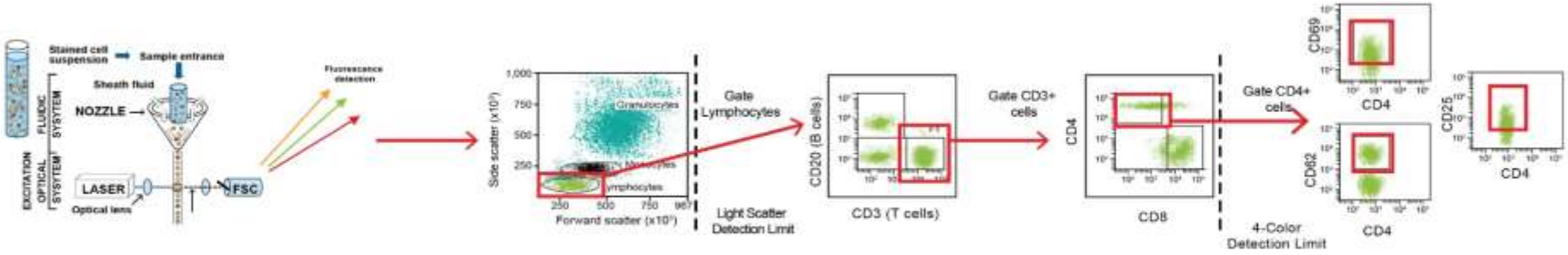
Flow Cytometry Overview

- Flow cytometry measures properties of individual particles, typically cells, passing in single file between a laser and a detector[1]
  - Light scatter conveys particle size and internal complexity[1]
  - Light emission from fluorescently labelled antibodies or dyes[1]
  - Capability to sort cells based on phenotype[2]
- Sensitivity and specificity improves with more lasers and detectors[3]



1. Brown M, et al. *Clin Chem*. 2000;46:1221-1229; Picot J, et al. *Cytotechnology*. 2012;64:109-130; 3. Chattopadhyay P, et al. *Methods*. 2012;57:251-258.

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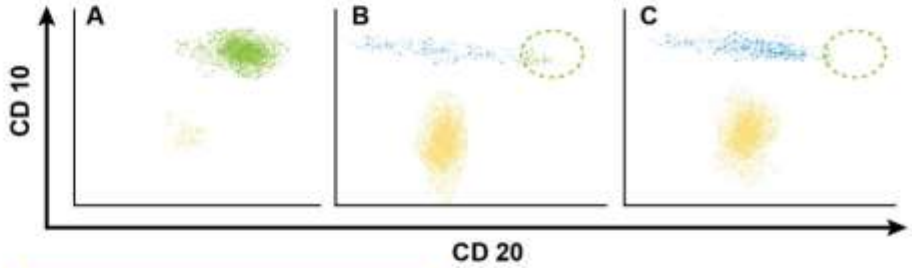
Flow Cytometry for MRD Detection

- Use of specific molecular or immunophenotypic markers and size to distinguish between malignant and normal hematopoietic cells[1]
- Establishment of standardized vs patient-specific antibody combinations[2]



A) MRD+ patient, pre-chemotherapy
B) MRD+ patient, post-chemotherapy
C) MRD- patient

Recommended 6-color flow cytometry panel for MM[3,a]

Tube 1	Tube 2
CD27	CD56
CD81	CD117
CD19	CD19
CD38	CD38
CD138	CD138
CD45	CD45

Examples of Markers Used in B-cell ALL[2,4]

CD10
CD19
CD34
CD38
CD45
CD58
CD123

[a] 8-color flow cytometry also requires 2 tubes.

1. Bruggemann M, et al. *Blood*. 2012;29:4470-4481; 2. Rocha JM, et al. *Mediterr J Hematol Infect Dis*. 2016;8:e2016024; 3. Mailankody S, et al. *Nat Rev Clin Oncol*. 2015;12:286-295; 4. Fossat C, et al. *Cytometry B Clin Cytom*. 2015;88:21-29. Image adapted from Hauwel, *Swiss Med Wkly*. 2014;144:w13907.

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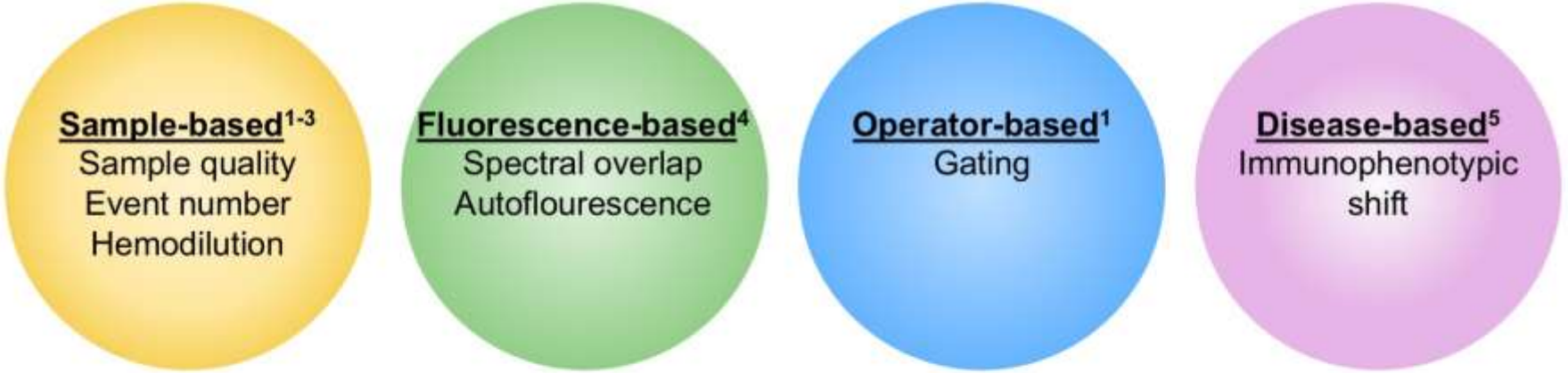
Sources of Error in Flow Cytometric Analysis



1. van Dongen JJ, et al. *Blood*. 2015;125:3996-4009; 2. Loken MR, et al. *Cytometry B Clin Cytom*. 2009;76:27-36; 3. Virgo PF, Gibbs GJ. *Ann Clin Biochem*. 2012;49:17-28; 4. Hulspas R, et al. *Cytometry B Clin Cytom*. 2009;76:335-364; 5. Bruggemann M, et al. *Blood*. 2012;29:4470-4481.

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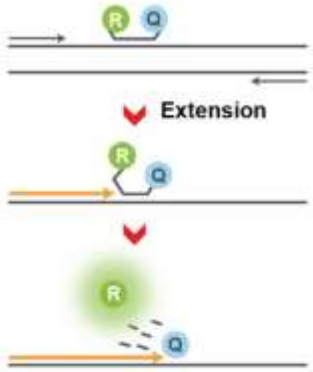
Quantitative Real-Time PCR (qPCR)

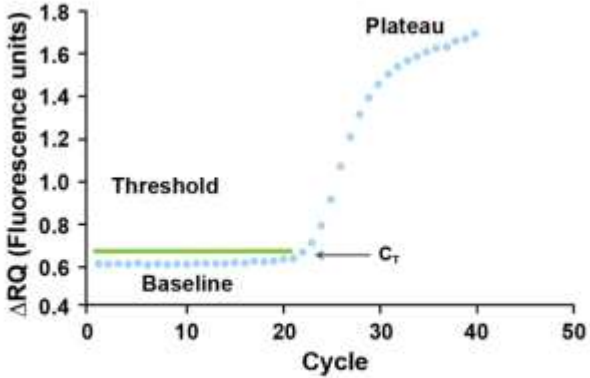
- Standard (qualitative) PCR determines presence or absence of mutated gene of interest[1,2]
- qPCR allows for estimation of tumor burden (frequency)[2]
  - Relies on a hybridization probe labeled with 2 different fluorescent dyes[3]
    - Reporter dye/Quencher dye
  - Comparison to a standard curve allows quantitation

During annealing, the hydrolysis probe binds to the target sequence



During extension, the probe is partially displaced and the reporter is cleaved. The free reporter fluoresces

An amplification curve is generated



1. Bruggemann M, et al. *Blood*. 2012;29:4470-4481; 2. Rocha JM, et al. *Mediterr J Hematol Infect Dis*. 2016;8:e2016024; 3. Held CA, et al. *Genome Res*. 1996;6:986-994.

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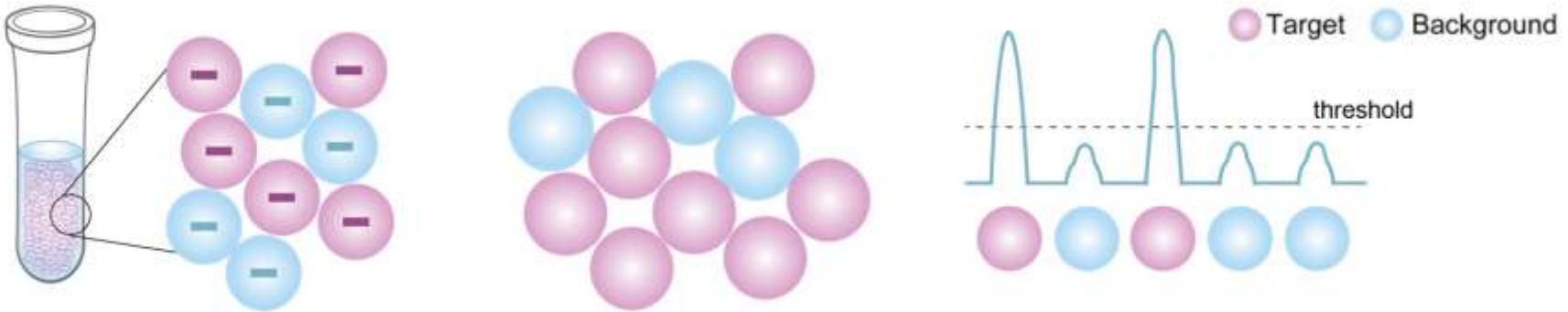
Alternative Approaches to Disease Quantitation: Droplet Digital PCR (ddPCR)

- Sample DNA is partitioned into ~20,000 droplets, allowing multiple parallel reactions[1]
- Provides quantitation without the need for standard curves[2]
- Similar sensitivity and accuracy to qPCR[2,3]
  - 88% sample concordance in ALL[2] and strong concordance in pooled MM, MCL, and FL samples[3] with correlation of coefficient of 0.94

Partitioning → **Amplification** → **Detection**

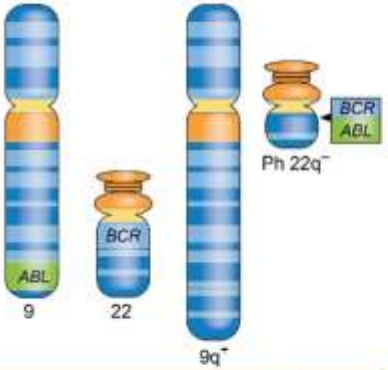


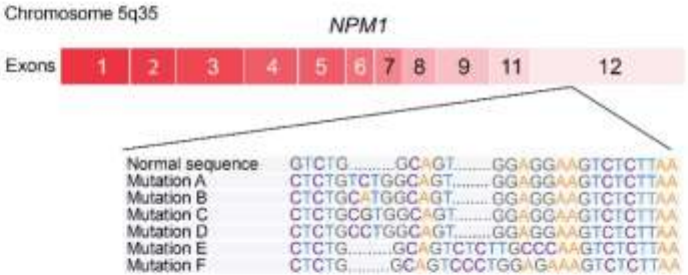
MCL, mantle cell lymphoma.

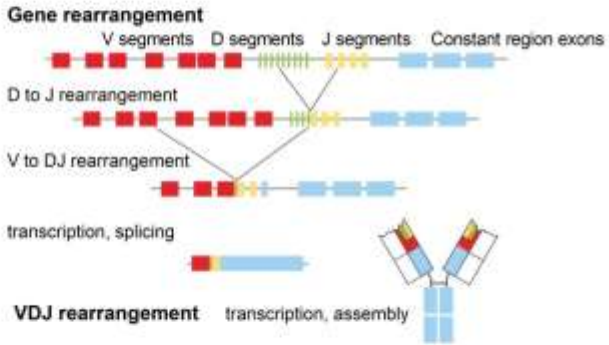
1. Hindson BJ, et al. *Anal Chem*. 2011;83:8604-8610; 2. Starza ID, et al. *Br J Haematol*. 2016;174:541-549; 3. Drandi D, et al. *J Mol Diagn*. 2015;17:652-660.

qPCR in MRD Detection

- qPCR has the capability to detect:







Gene fusions[1,2]

Point mutations/Insertions/Deletions[3,4]

Gene rearrangements[1]

1. van Dongen JJ, et al. *Blood*. 2015;125:3996-4009; 2. Lydon N. *Nat Med*. 2009;15:1153-1157; 3. Ivey A, et al. *N Engl J Med*. 2016;374:422-433; 4. Licinio MA, et al. *Rev Bras Hematol Hemoter*. 2010;32:476-481.

Emerging Platforms: Next-Generation Sequencing (NGS)

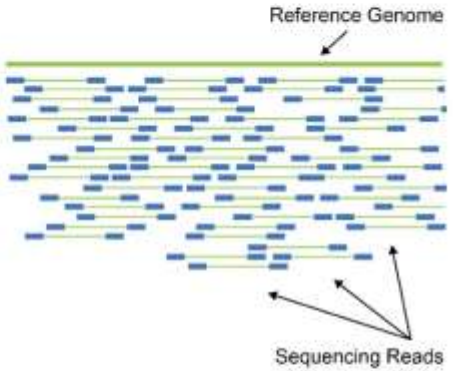
- Sensitivity[1]: 10^{-5} – 10^{-6}
  - High concordance with both flow cytometry and qPCR[1,2]

Methods[3]

- Sequencing by synthesis
- Pyrosequencing
- Sequencing by ligation
- Nanopore

Terms[4,5]

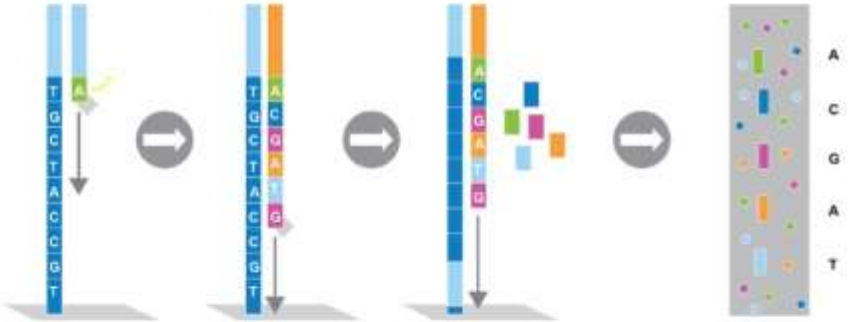
- Library= a collection of DNA or cDNA for sequencing
- Adapters- synthetic DNA added during library generation
- Barcodes = unique DNA sequences that can be added to individual libraries for multiplexing
- Read=base pair information generated by sequencing
- Read length = number of bases sequenced
- Depth of Coverage= the number of distinct reads of a given DNA sequence



1. Faham M, et al. *Blood*. 2012;120:5173-5180; 2. Kotrova M, et al. *Blood*. 2015;126:1045-1047; 3. Levy SE, et al. *Annu Rev Genom Hum Genet*. 2016;17:95-115; 4. Seibold A. eSeminar presentation; October 8, 2013. Available at: http://slideshowes.com/doc/1554921/back-to-the-basics--next-generation-sequencing-101. Accessed November 17, 2016; 5. Robasky K, et al. *Nat Rev Genet*. 2014;15:56-62.

NGS Methods: Sequencing by Synthesis

- Nucleotides are passed over immobilized sample DNA fragments[1]
- DNA synthesis occurs one base at a time[1]
- Incorporation of nucleotide generates a signal[1]
- Examples:[1,2]
  - Fluorescently tagged nucleotides
  - Ion Torrent: nucleotide binding triggers a pH change



1. Moorthie S, et al. *Hugo J.* 2011;5:1-12; 2. Levy SE, et al. *Annu Rev Genom Hum Genet.* 2016;17:95-115.

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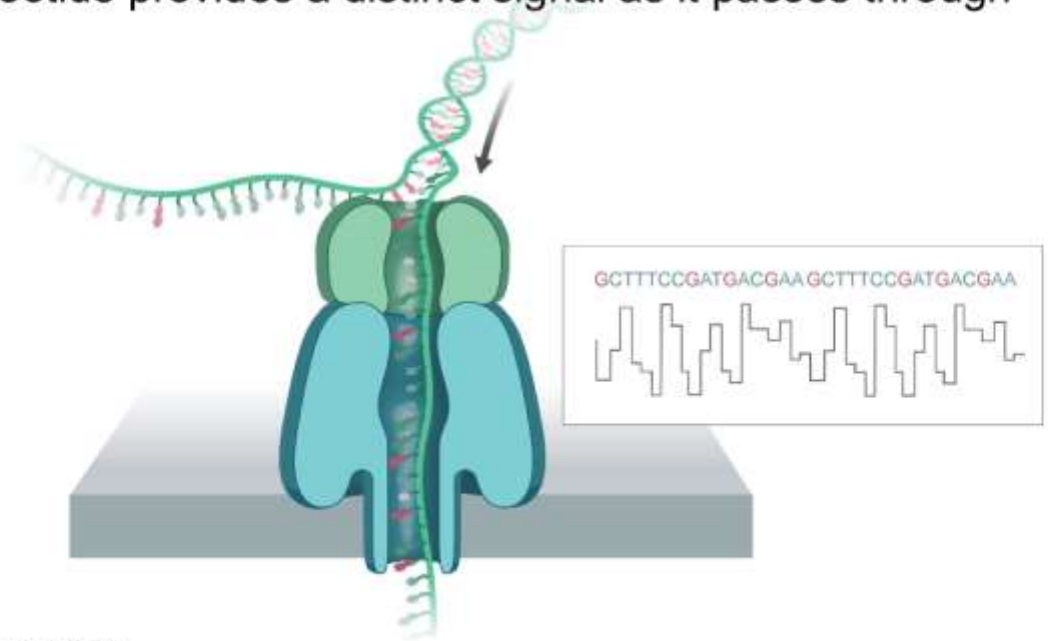
NGS Methods: Nanopore[1]

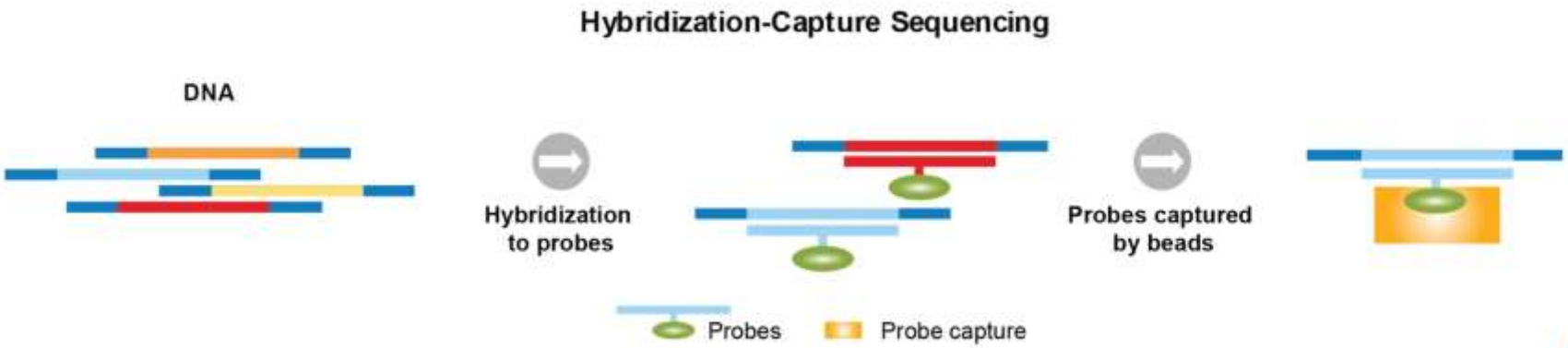
- Voltage current is applied across a nanopore
- DNA is drawn through the nanopore
  - Each nucleotide provides a distinct signal as it passes through



1. Lu H, et al. *Genomics Proteomics Bioinformatics*. 2016;14:265-279.

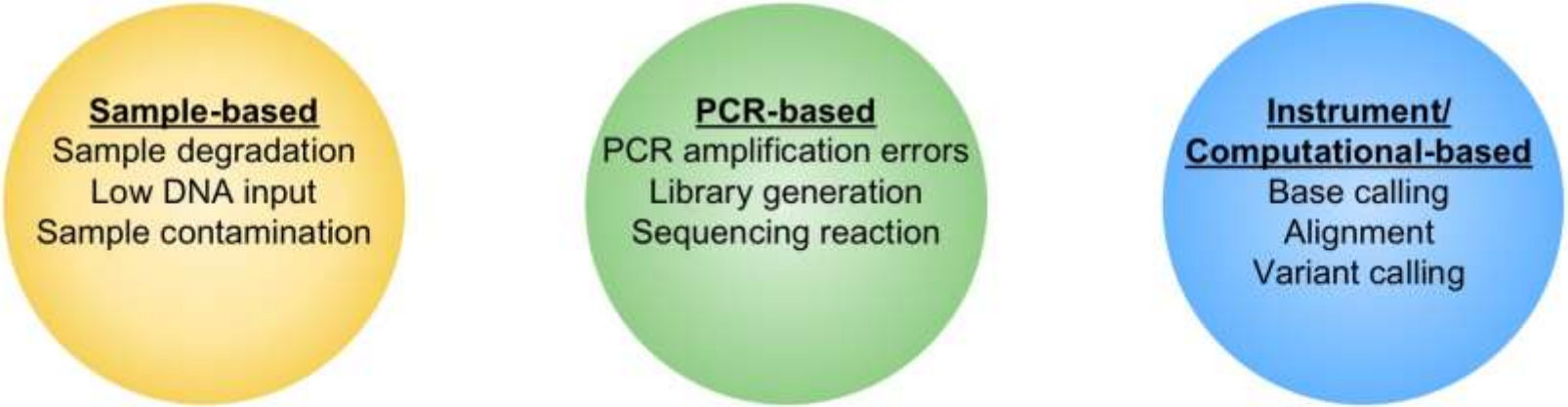
Amplicon vs Array-Based Sequencing

- During NGS, hundreds of millions of DNA segments are sequenced in parallel[1]
- Targeted sequencing focuses only on genes of interest[2]
  - Hybridization-capture: gene specific hybridization probes[2]
  - Amplicon-based: gene specific primer sets[3]
- Multi-array panels identify a broad scope of the genetic mutations present[4]



1. Lin X, et al. *Hear Res*. 2012;288:67-76; 2. Bodi K, et al. *J Biomol Tech*. 2013;24:73-86; 3. Porreca GJ, et al. *Nat Methods*. 2007;4:931-936; 4. Luthra R, et al. *Haematologica*. 2014;99:465-473.

Sources of Error in NGS Based Analysis



Robasky K, et al. *Nat Rev Genet*. 2014;15:56-62.

MRD Detection Methods: Important Considerations

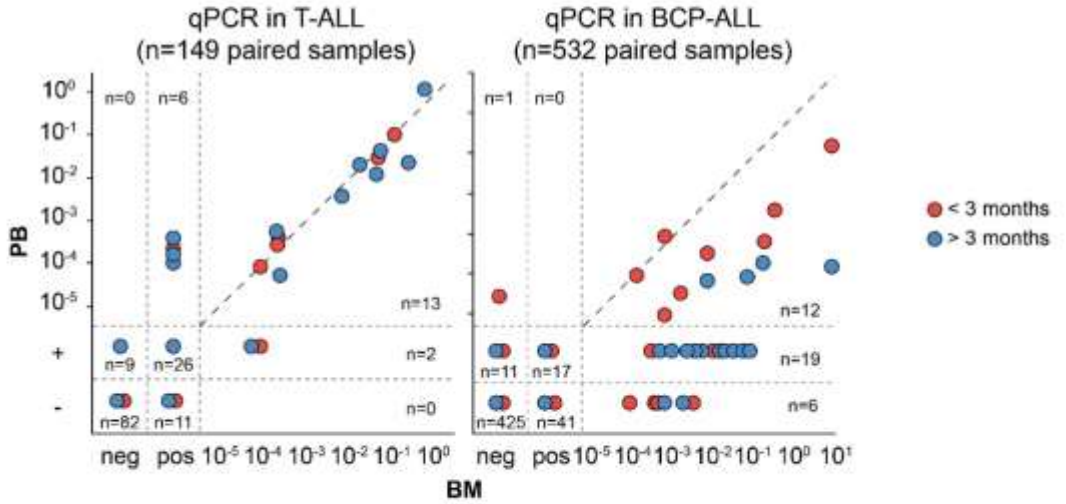
	Important Considerations	
Flow Cytometry[1]	• Rapid • Applicable in most patients	• False positives due to similarities in phenotypes • Less sensitive than other techniques • Limited standardization between laboratories
qPCR[1,2]	• High sensitivity: 10^{-4} – 10^{-5} • High standardization and automation	• False negatives due to clonal evolution
NGS[2-4]	• High sensitivity: 10^{-5} – 10^{-6}	• Limited standardization across laboratories • Complex bioinformatics

1. Bruggemann M, et al. *Blood*. 2012;120:4470-4481; 2. Rocha JM, et al. *Mediterr J Hematol Infect Dis*. 2016;8:e2016024; 3. Van Dongen JJ, et al. *Blood*. 2015;125:3996-4009; 4. Faham M, et al. *Blood*. 2012;120:5173-5180.

MRD Testing: Bone Marrow (BM) vs. Peripheral Blood (PB)

- While BM samples are frequently used, PB as a less invasive sample option has been proposed[1]
- Results vary by disease

Similar Detection Levels	BM More Sensitive than PB
T-cell ALL[1]	B-cell ALL[1,2]
AML[3]	MM[4]
CML[5]	
FL[6,a]	
MCL[6]	
CLL[7,b]	



[a] A previous study using less sensitive testing methods reported BM having superior detection limits; [b] In patients not undergoing treatment or chemotherapy. Advantages of PB or BM may vary by treatment.

1. Van Dongen JJ, et al. *Blood*. 2015;125:3996-4009; 2. Fossat C, et al. *Cytometry B Clin Cytom*. 2015;88:21-29; 3. Buccisano F, et al. *Blood*. 2012;119:332-341; 4. Korthals M, et al. *Biol Blood Marrow Transplant*. 2013;19:1109-1115; 5. Akard LP, et al. *Arch Pathol Lab Med*. 2014;138:1186-1192; 6. Sorenson CD, et al. *J Immunol Methods*. 2014;406:131-136; 7. Rawstron AC, Hillmen P. *Curr Hemat Malig Rep*. 2008;3:47-53. Image adapted from Van Dongen JJ, et al. *Blood*. 2015;125:3996-4009.

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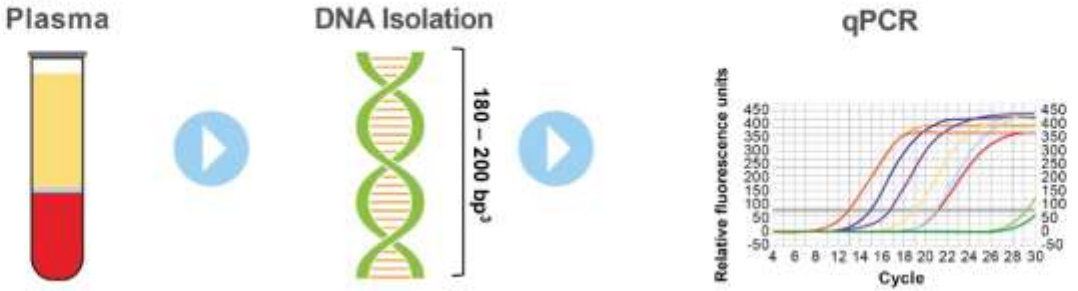
Developing Detection Methods: Cell-Free Circulating Tumor DNA

- Shedding of apoptotic or necrotic cell DNA fragments into the bloodstream is physiologic[1]
- In patients with cancer, a large fraction of cell-free DNA is tumor derived and termed circulating tumor (ct) DNA[1,2]
- ctDNA has use in a variety of applications:[1]
  - Detecting specific mutations
  - Assessing tumor burden
  - Monitoring therapy responses
  - MRD surveillance



bp, base pairs.

1. Qin Z, et al. *Chin J Cancer*. 2016;35:36; 2. Bettegowda C, et al. *Sci Transl Med*. 2014;6:224ra24; 3. Diaz LA, Bardelli A. *J Clin Oncol*. 2014;32:579-586.

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Established and Exploratory Uses of MRD in Disease Management

MRD in Patient Monitoring

- Once a patient achieves a complete remission/response, regular monitoring for relapse is important[1]
- Patient monitoring schedules provide specific time points at which MRD can be used to assess a patient's response to treatment and predict disease progression[1,2]
- Example: The European LeukemiaNet guidelines for CML[2]
  - Monitoring can be performed using a molecular or cytogenetic test, or both

MMR, major molecular response.

1. Bruggemann M, et al. *Blood*. 2012;120:4470-4481; 2. Baccarani M, et al. *Blood*. 2013;122:872-884.

Use of MRD in Heme Malignancies

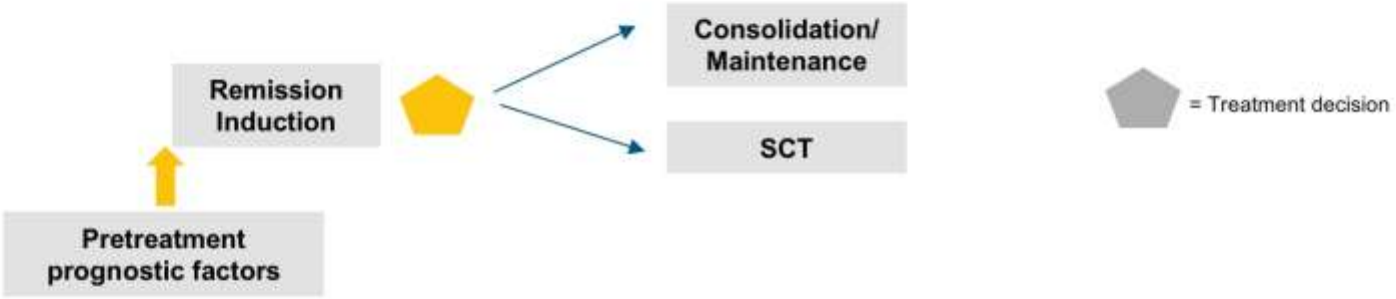
MRD has been used for risk stratification and in guiding treatment decisions

Disease	Patient Population	MRD Detection Method
ALL[1]	Adults, post-induction	qPCR
ALL[2]	Children, newly diagnosed	Flow cytometry and/or qPCR
AML[3]	*NPM1*-mutated AML	qPCR
AML[4]	Children with mainly de novo AML	Flow cytometry
MM[6]	Post-ASCT	Flow cytometry
CLL[7]	Post-chemotherapy	Flow cytometry

1. Bruggemann M, et al. *Blood*. 2006;107:1116-1123; 2. Pui C-H, et al. *N Eng J Med*; 2009:2730-2741; 3. Ivey A, et al. *N Engl J Med*. 2016;374:422-433; 4. Rubnitz JE, et al. *Lancet Oncol*. 2010;11:543-552; 5. Paiva B, et al. *Blood*. 2008;112:4017-4023; 7. Ysebaert L, et al. *Leukemia* 2010;24:1310-1316

MRD in Choosing Post-Induction Treatment Regimens

- Treatment decisions based on MRD may allow for the personalization of therapy regimens[1,2]



SCT, stem cell transplantation.

1. Buccisano F, et al. Blood. 2012;119:332-341; 2. Hauwel M, et al. Swiss Med Wkly. 2014;144:w13907. Diagram adapted from Bruggemann M, et al. Blood. 2012;120:4470-4481.

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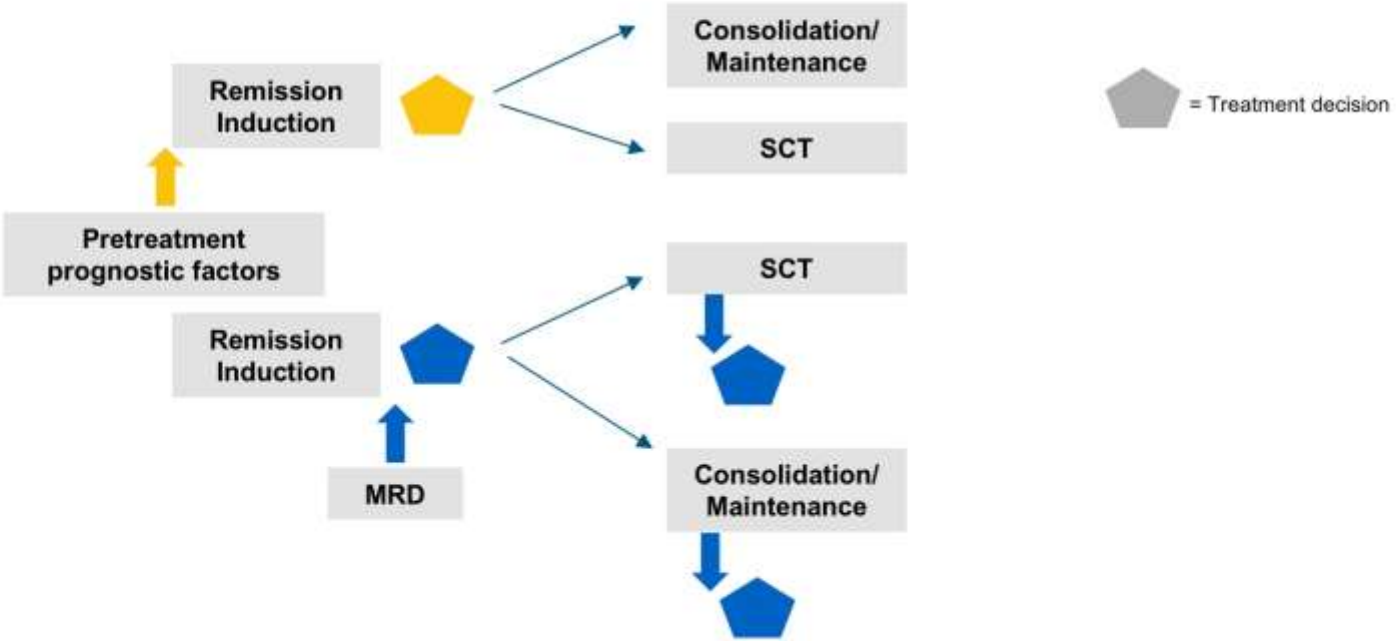

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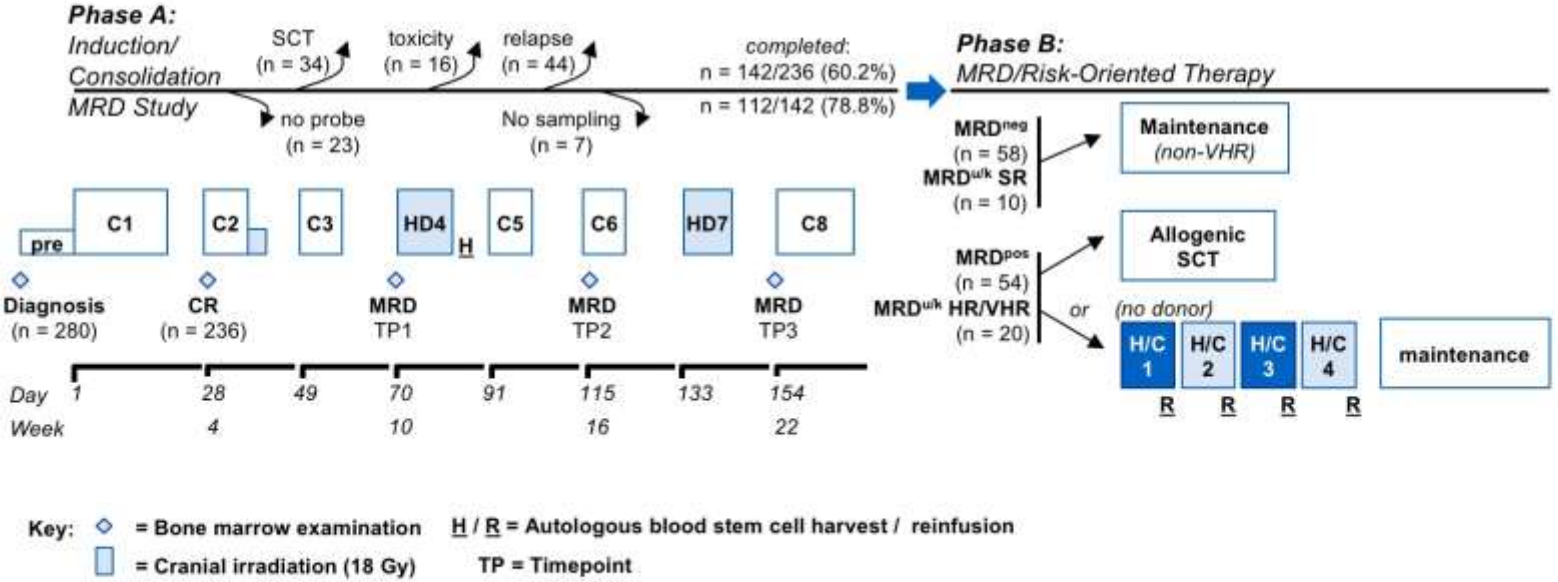
MRD Can Identify Patients with ALL for SCT

- MRD status after induction determined treatment
  - MRD negative: maintenance therapy
  - MRD positive: hyper-fractionated cycle treatment (H/C) and SCT



Key: ◇ = Bone marrow examination H / R = Autologous blood stem cell harvest / reinfusion
▯ = Cranial irradiation (18 Gy) TP = Timepoint

HR, high risk; SR, standard risk; VHR, very high risk.
Bassan R, et al. *Blood*. 2009;113:4153-4162.

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MRD as a Surrogate Clinical Endpoint

- Traditional clinical endpoints such as OS are the "gold standard" primary endpoint[1]
- Surrogate endpoints can predict clinical benefit without directly measuring clinical benefit itself[2]
  - Potential to expedite drug development
- Since MRD status may correlate with patient outcomes and can be measured at earlier time points, it has potential for use as a surrogate endpoint[2]

OS, overall survival.

1. Bruggemann M, et al. *Blood*. 2012;120:4470-4481; 2. Gormley NJ, et al. *JAMA Oncol*. 2016. Published online ahead of print; September 15, 2016. doi:10.11/jamaoncol.2016.3112.

MRD as a Surrogate Clinical Endpoint (cont.)

- Challenges:[1]
  - Standardization of assays
  - Determination of proper MRD threshold
  - Optimizing measurement time points
- CML: accelerated US FDA approval for tyrosine kinase inhibitor was based on BCR-ABL transcript levels detected by qPCR[2,3]
- Workshops have been held by the US FDA to discuss potential adoption of MRD as a surrogate endpoint for ALL, CLL, AML, and MM[1,4]
- MRD is currently approved as a surrogate endpoint in CLL by the EMA[5]

EMA, European Medicines Agency; US FDA, United States Food and Drug Administration.

1. Gormley NJ, et al. *JAMA Oncol*. 2016. Published online ahead of print; September 15, 2016. doi:10.11/jamaoncol.2016.3112; 2. Saglio G, et al. *N Engl J Med*. 2010;362:2251-2259; 3. US FDA News Release. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm216181.htm, accessed October 21, 2016; 4. Gormley NJ, et al. *Cytometry B Clin Cytom*. 2016;90:73; 5. European Medicines Agency. www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/12/WC500179047.pdf. Accessed November 18, 2016.

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Summary

MRD Summary

- MRD is the presence of malignant cells under the detection limit of conventional methods[1]
- Strong prognostic indicator of patient outcomes[2]
- Potential for use in guiding treatment decisions[3]
  - Intensity of therapy
  - Appropriateness of SCT
- Currently measured using flow cytometry or qPCR[2]
  - Newer testing methods are emerging
- MRD is currently being evaluated as a potential surrogate endpoint, and may have the potential to replace or augment morphological CR as a response criteria in certain hematologic malignancies[4]

1. Inaba H, et al. *Lancet*. 2013;381:1943-1955; 2. Van Dongen JJ, et al. *Blood*. 2015;125:3996-4009; 3. Buccisano F, et al. *Blood*. 2012;119:332-341; 4. Gormley NJ, et al. *JAMA Oncol*. 2016. Published online ahead of print, September 15, 2016. doi:10.11/jamaoncol.2016.3112.

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