Baseline Genomic Testing in Lymphoma: Are We There Yet?

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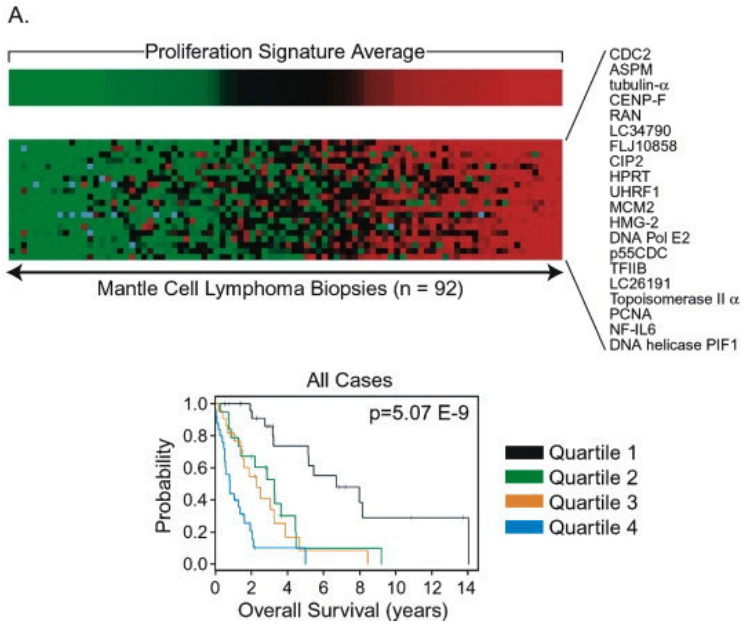
Disclosure

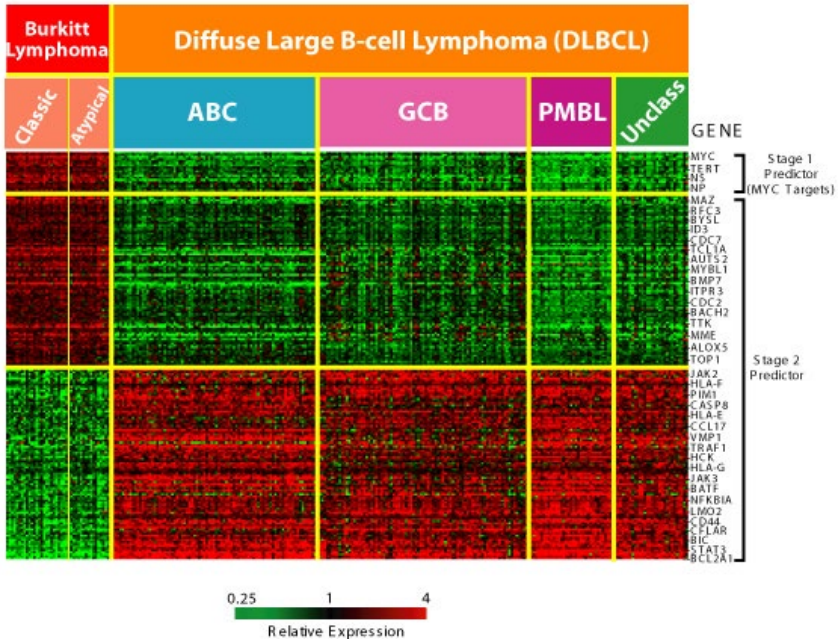
No disclosures

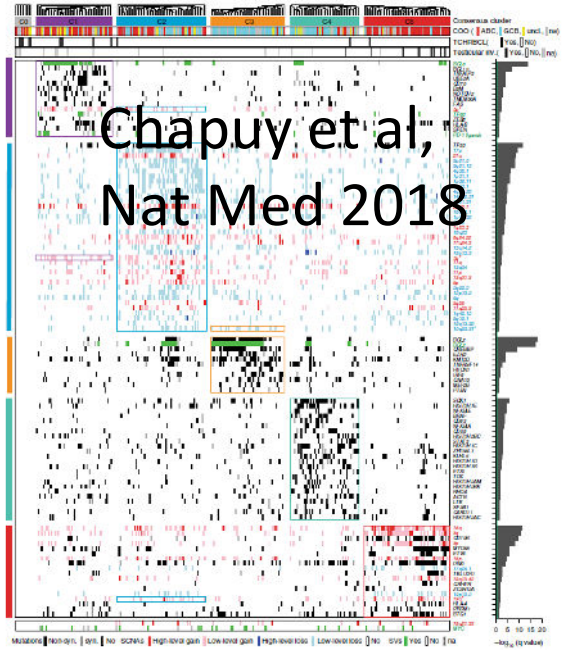
Genomic approaches have been the driver of discoveries for the past two decades



Rosenwald et al,
Cancer Cell 2003

Dave et al, NEJM 2006

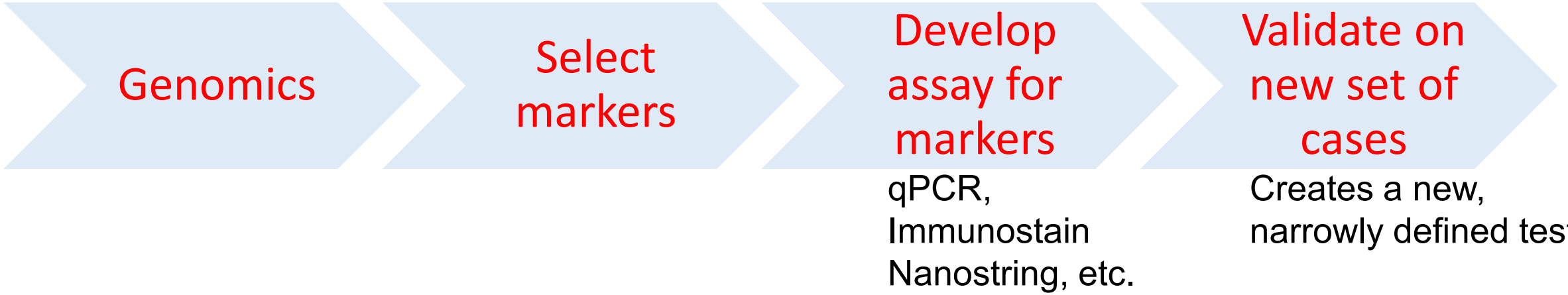




Chapuy et al,
Nat Med 2018

Why is translation of genomic findings lacking?

The paradigm for clinical translation is
Complex, slow and expensive.



NGS could potentially replace the need for multiple types of tests

Current Guidelines for Lymphoma: Mutations

Feature	Gene	Entity	Essential/UUCC
MYD88 L265P	MYD88	Waldenstrom Macroglobulinemia/Lymphoplasmacytic Lymphoma	Essential
TP53	TP53	Mantle Cell Lymphoma	Essential
BRAF V600E	BRAF	Hairy Cell Leukemia	UUCC
BTK	BTK	Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma	UUCC
CXCR4	CXCR4	Waldenstrom Macroglobulinemia/Lymphoplasmacytic Lymphoma	UUCC

Current Guidelines for Lymphoma: Rearrangements

Feature	Gene	Entity	Essential/UUCC
PDGFRB rearrangement	PDGFRB	Acute Lymphoblastic Leukemia (ALL)	Essential
BCL2 rearrangement	IGH-BCL2	Lymphoma	Essential
BCL6 rearrangement	IGH-BCL6	Lymphoma	Essential
MYC rearrangement	IGH-MYC	Lymphoma	Essential
t(11;14)	IGH-CCND1	Lymphoma	Essential
IRF4/MUM1 rearrangement	IRF4-MUM1	DLBCL	UUCC
DUSP22-IRF4 rearrangement	DUSP22-IRF4	Peripheral T-Cell Lymphomas	UUCC
TP63 rearrangement	TP63	Peripheral T-Cell Lymphomas	UUCC

Current Guidelines for Lymphoma: Expression

Feature	Entity	Essential/UUCC
Annexin A1	Splenic Marginal Zone Lymphoma	Essential
BCL2	Follicular Lymphoma (grade 1-2)	Essential
BCL6	Follicular Lymphoma (grade 1-2)	Essential
CD10	Follicular Lymphoma (grade 1-2)	Essential
CD103	Splenic Marginal Zone Lymphoma	Essential
CD11C	Hairy Cell Leukemia	Essential
CD13	Lymphoblastic Lymphoma	Essential
CD163	Erdheim-Chester Disease	Essential
CD19	Follicular Lymphoma (grade 1-2)	Essential
CD207	Langerhans Cell Histiocytosis	Essential
CD21	Follicular Lymphoma (grade 1-2)	Essential
CD22	Lymphoblastic Lymphoma	Essential
CD23	Follicular Lymphoma (grade 1-2)	Essential
CD279	Peripheral T-Cell Lymphomas	Essential
CD45	Diffuse Large B-Cell Lymphoma	Essential
EBV-EBNA2	Post-Transplant Lymphoproliferative Disorders	Essential
EBV-LMP1	Post-Transplant Lymphoproliferative Disorders	Essential
LEF1	Mantle Cell Lymphoma	UUCC
PAX5	Gray Zone Lymphoma	UUCC
PDCD1	Mycosis Fungoides/Sezary Syndrome	UUCC
Perforin	Peripheral T-Cell Lymphomas	UUCC
TIA-1	Post-Transplant Lymphoproliferative Disorders	UUCC

Typical final diagnosis report

Genetic Tests (DNA)
FISH BCL2
FISH BCL6
FISH MYC x 2

Expression Tests (Protein/RNA)
CD10 x 2
PAX5
BCL2
BCL6
IRF4/MUM1
MYC
CD21
KI67
EBER

13 Different tests

Description: The nodal architecture is effaced by a diffuse population of intermediate to large cells with somewhat dispersed chromatin, round nuclear contours and scant cytoplasm. Flow cytometric studies previously performed on this sample at [redacted] were reported to show a CD10-positive B-cell non-Hodgkin lymphoma (see report P18-340). A paraffin block was obtained from the referring institution and additional levels cut at [redacted] for additional immunohistochemistry. Immunostains performed at [redacted] showed numerous PAX-5-positive B-cells that coexpress CD10, Bcl-6 and MYC (100%, strong). The B-cells are negative for MUM-1. A Bcl-2 stain is variable with weak staining in a subset of the neoplastic cells (approximately 20-30%) using clone 124, but Bcl-2 clone EP36 shows strong diffuse staining throughout (100%). A CD21 stain is negative. A Ki-67 stain is positive in approximately 70% of nuclei. An EBER in situ hybridization stain is negative.

FISH studies were positive for a BCL2 translocation and negative for BCL6 and MYC translocations. The findings in this case are best classified as a diffuse large B-cell lymphoma, not otherwise specified (2016 WHO classification)

Final Diagnosis

How can move genomics into routine clinical practice?

How can we…

- Get to the diagnosis faster (and cheaper)
- Empower clinicians and patients with complete information at the time of diagnosis (or relapse)
- Bridge the gap between discovery and translation

Need to Rethink Clinical Genomics

1. Need a simplified **Assay** for processing samples for DNA and RNAseq, needs to be FFPE compatible
2. Easy to use **Bioinformatics** software
3. Focus on diagnostic pathology

We sought to address these problems

What we developed

1. A new in house **Assay** for DNA and RNA sequencing: **Duoseq**

2. Comprehensive, **bioinformatics software** included with assay

3. Clinically validated readouts in collaboration with clinical labs

Duoseq--DNA & RNAseq: Sample to Report next day

Day 1: Kitted test at clinical lab

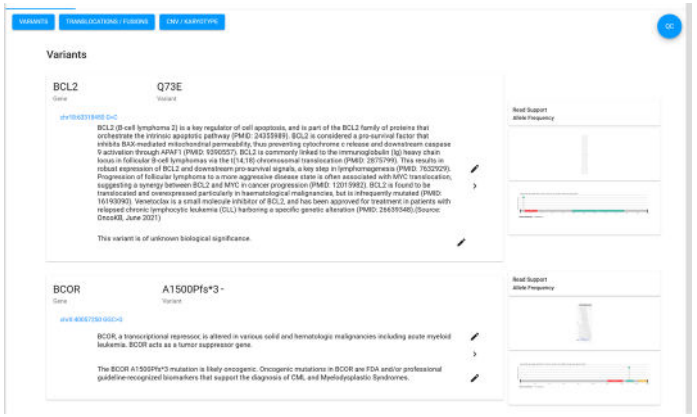
Perform Duoseq

Day 1: Overnight Sequencing

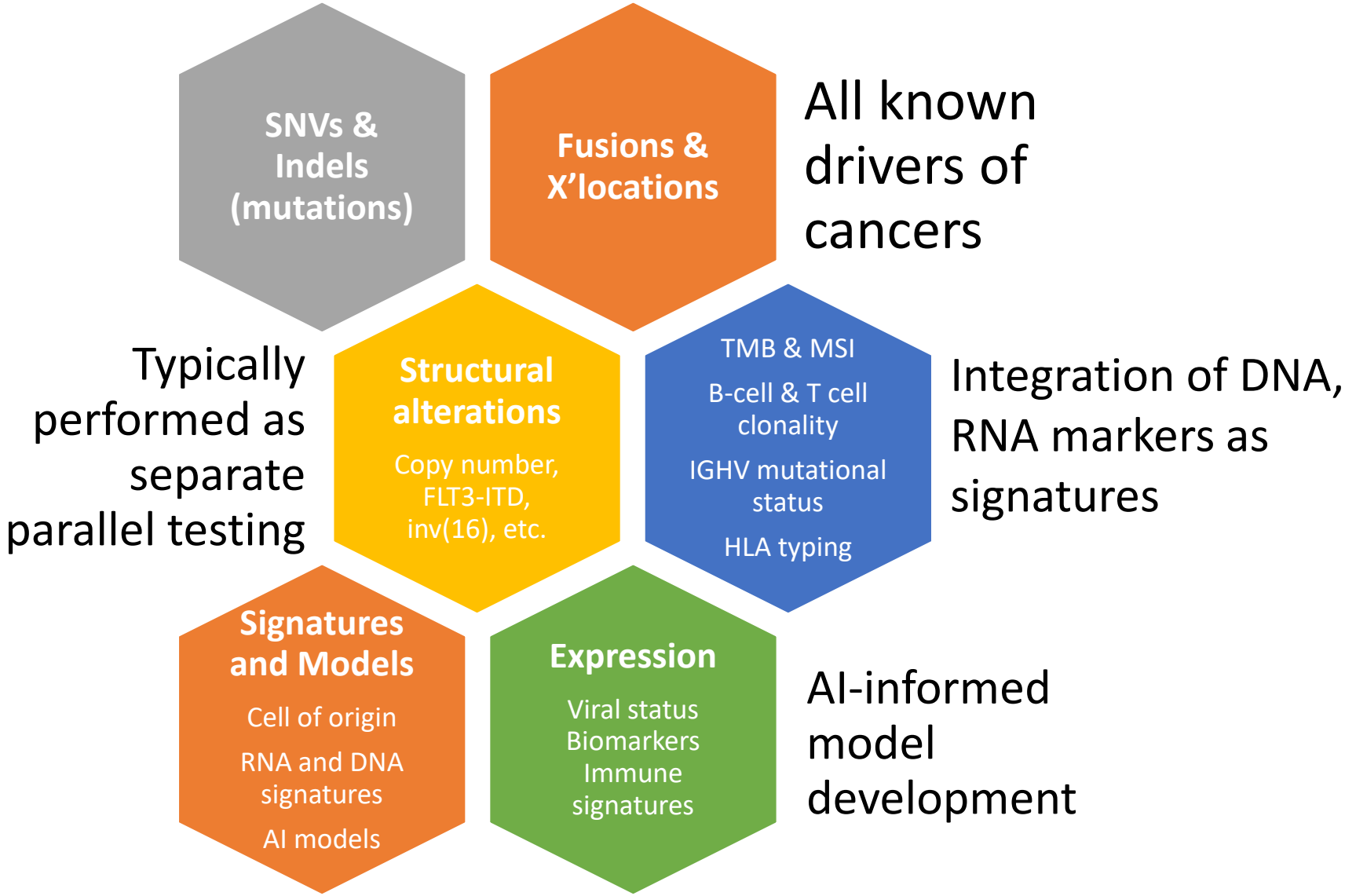
Sequencing

Day 2: Results in web-portal & EHR

Analysis and Reporting



Duoseq enables comprehensive readouts



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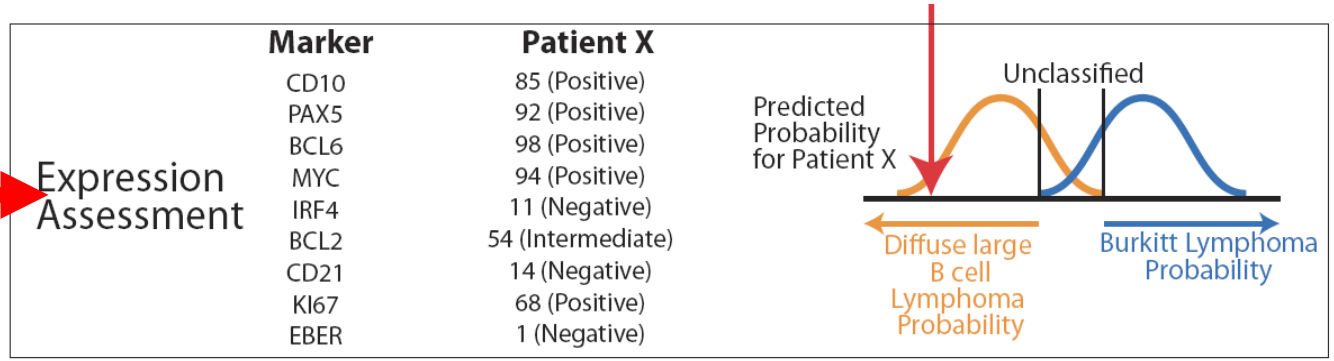
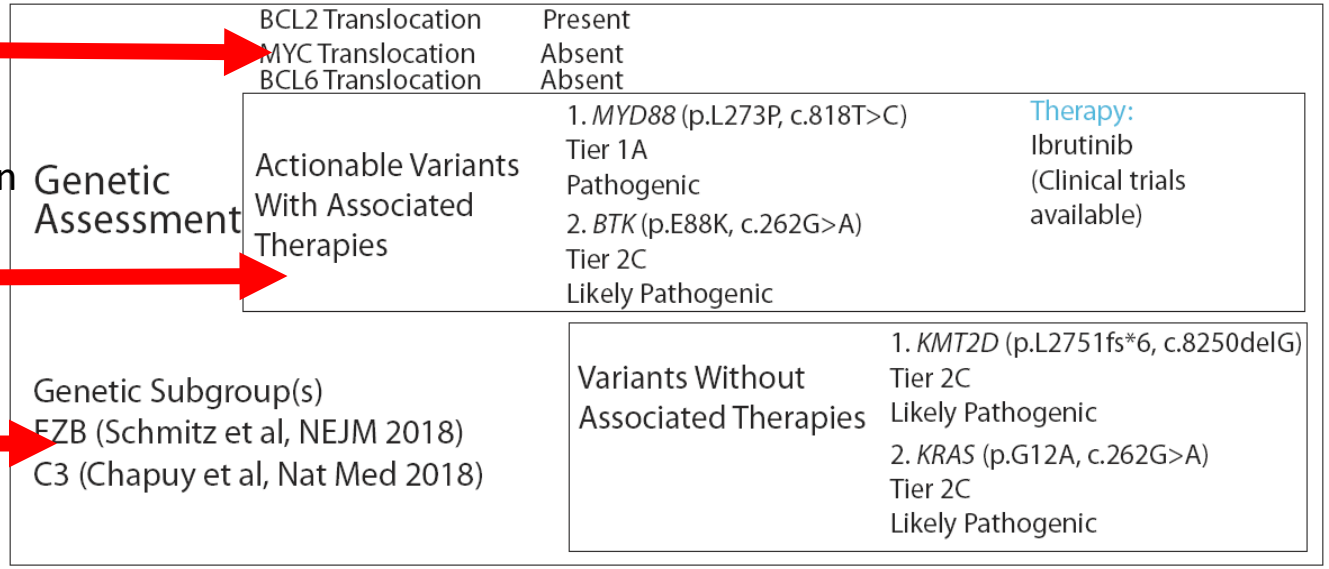
Duoseq Report

Measurements from RNA compared with large dataset

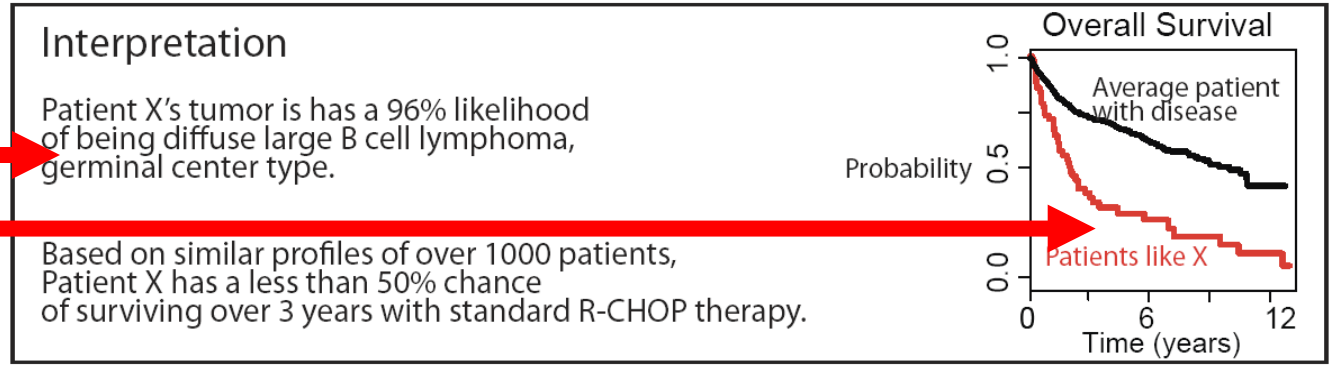


Translocation data



Up to date variant annotation reported using CAP/ASCO guidelines

Genetic subgroups and estimated survival



Clinical Validation Summary & Status of Test

153 FFPE samples with orthogonal validation at two clinical labs.

Component	SNVs	Indels	Translocations & Fusions
Total samples	132	132	153
PPV	98.2%	99.1%	95%
NPV	97.7%	96.6%	99.1%

Time-effective: Next-day results

Cost-effective: starts at ~$350

Now clinically and commercially available as Duoseq
Live at two hospitals (and growing)

Applications for Clinical Genomics in Lymphoma

Comprehensive Clinical Diagnostic—can replace routine clinical testing. In ANY clinical lab.

Biomarker Discovery and Translational Tool—can use for discovery of any combination of DNA and RNA markers that can be immediately deployed in the clinic

Companion Diagnostic: To identify patients for the right drug. Any combination of DNA and RNA markers. Clinic Ready.

MRD detection: Identify patients with early relapse or lack of response.

What the world needs now*

The value of baseline NGS in lymphoma is not that high—
based on current guidelines

It is time to rethink Baseline Genomic Testing in the clinic—
Done right, NGS, FISH and other routine clinical testing. In ANY clinical lab.

We need to empower clinicians and pathologists with data—really important to move data and results to the frontline of clinical care for design of the best studies and delivery of the best care.

Available for collaboration/clinical testing.

*With apologies to Burt Bacharach, Hal David & Jackie DeShannon

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Atlas of Blood Cancer Genomes Collaborators

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Duoseq team