ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI
SCIENZE MEDICHE E CHIRURGICHE

POLICLINICO DI
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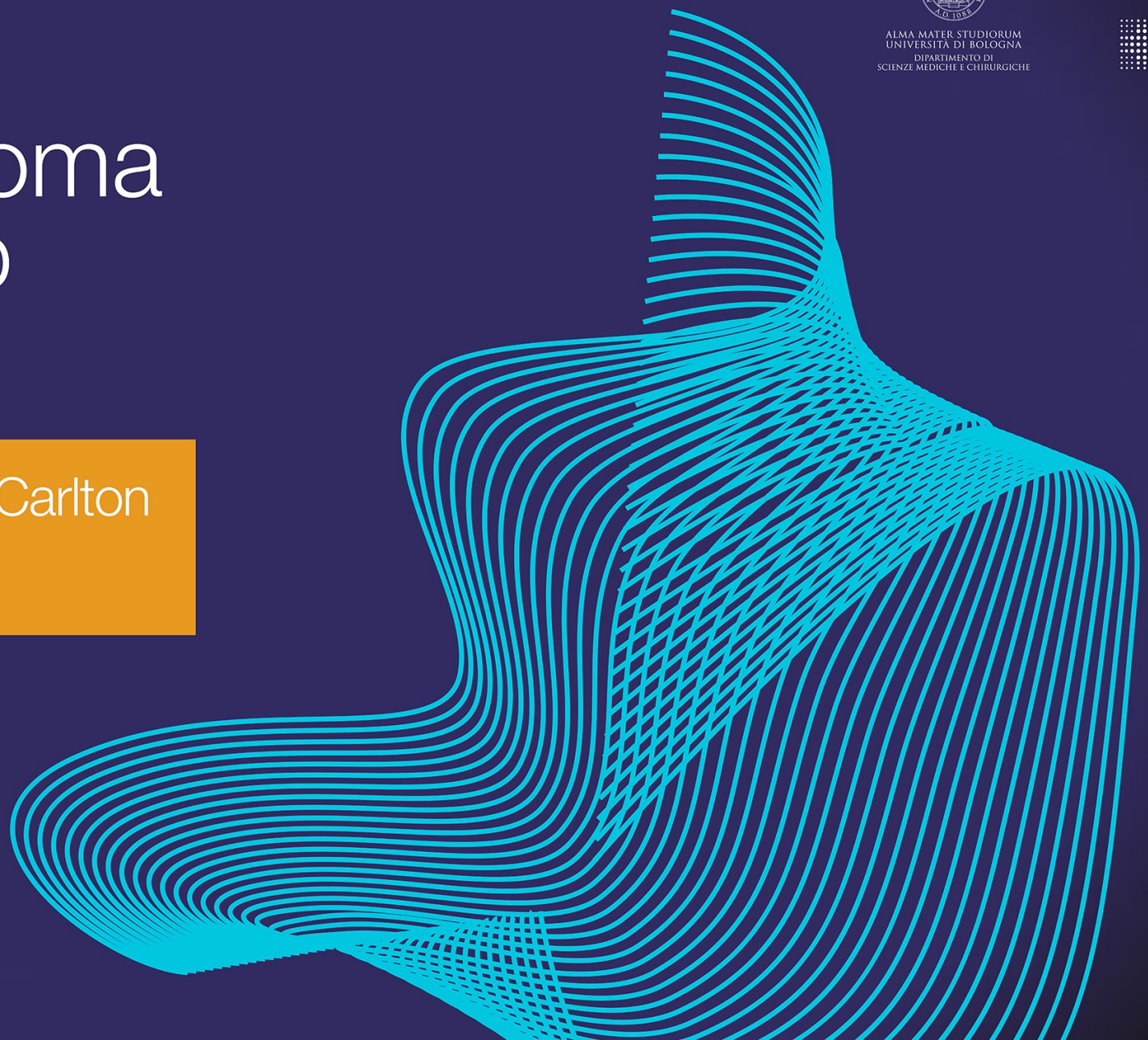
SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Bologna

Aggressive Lymphoma Workshop



Bologna, Royal Hotel Carlton

May 8-9, 2023

President: **Pier Luigi Zinzani**

Practical considerations for using genome sequencing data for patient selection

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BC CANCER
Provincial Health Services Authority

UBC
a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

Disclosures

Consulting: Abbvie, AstraZeneca, Incyte, Janssen

Research funding: Janssen, Roche/Genentech

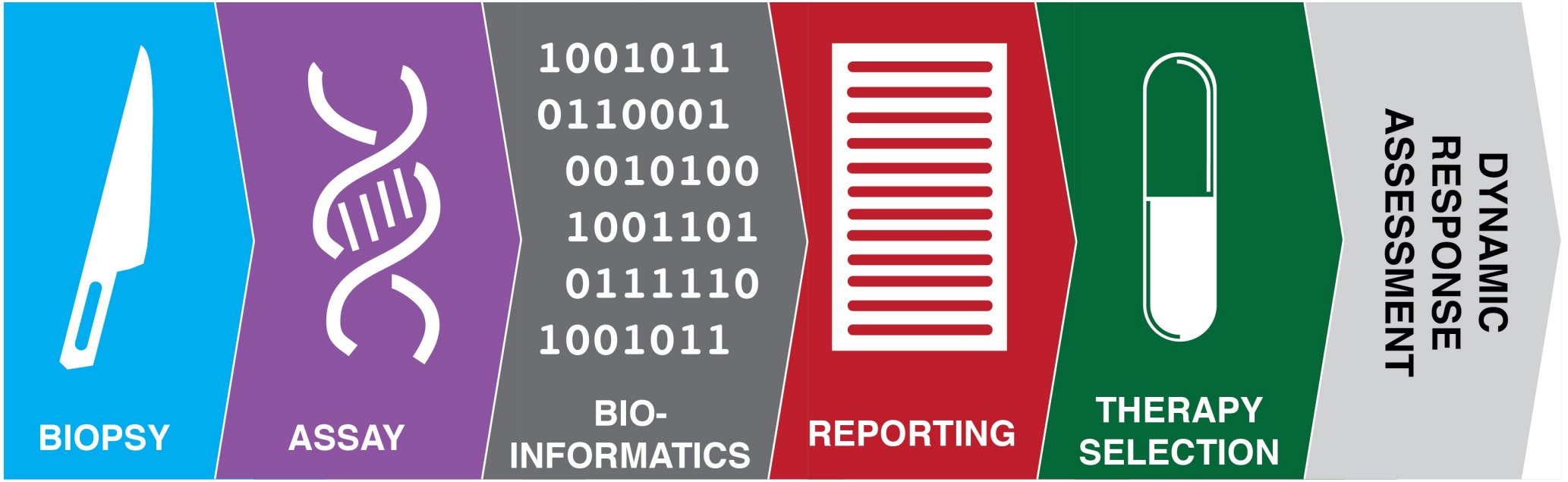
Patents: named inventor on patents related to using gene expression to identify subtypes of aggressive B-cell lymphomas – one of which is licensed to NanoString Technologies

In order to realize the promise of precision medicine in lymphoma, it needs to be shown that matching treatment to the tumour biology improves outcomes

The Challenge:

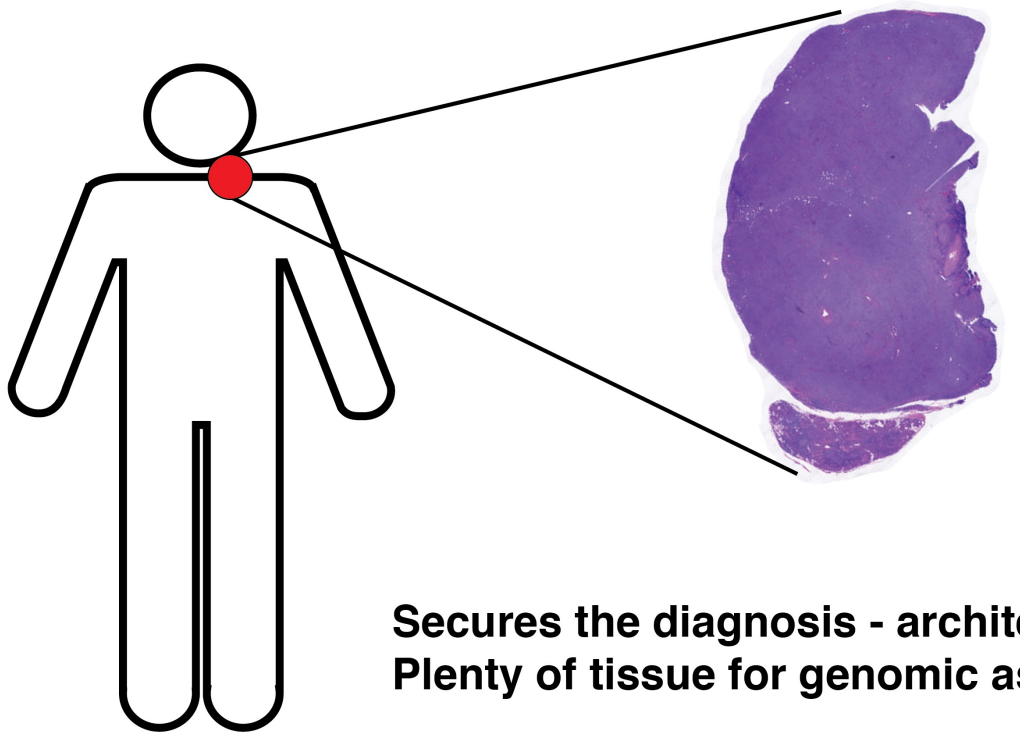
Providing timely and robust molecular analysis to guide rational therapy choice to support precision medicine trials in lymphoma and, ultimately, routine patient management

Outline: The components



Pathology – securing the diagnosis

An adequate biopsy



Incisional
Excisional

National Comprehensive Cancer Network®

GOOD SCIENCE
BETTER MEDICINE
BEST PRACTICE

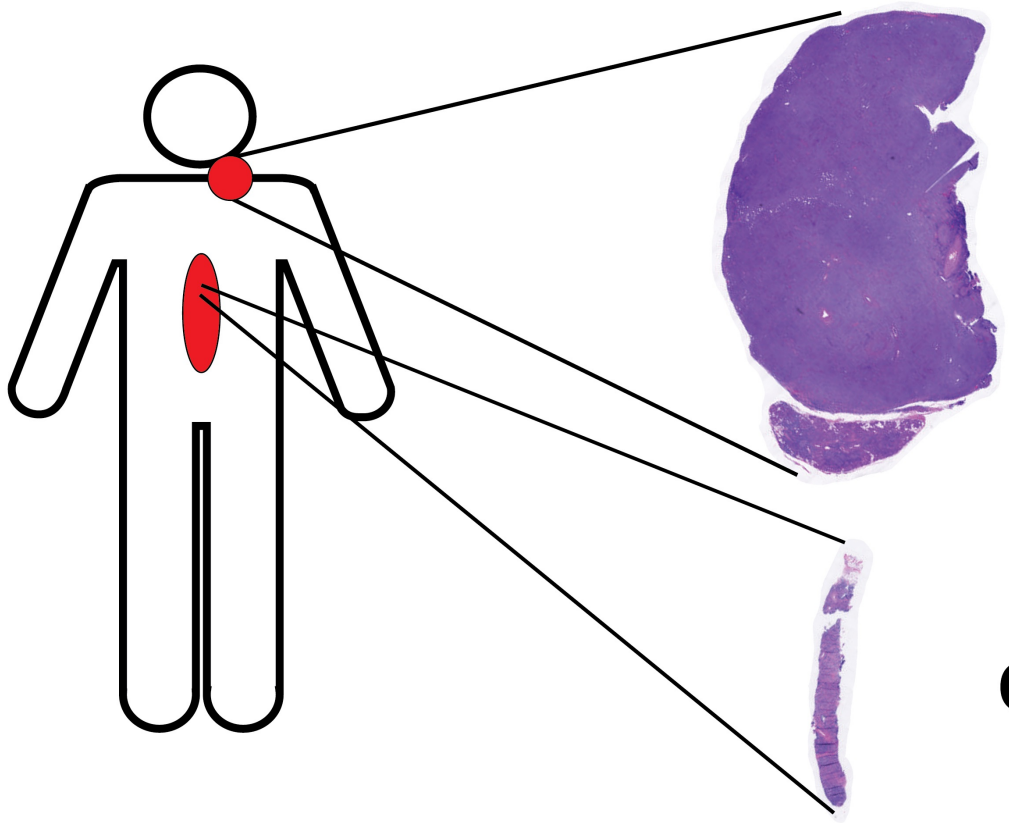
British Society for Haematology
Listening • Learning • Leading

Secures the diagnosis - architecture, IHC, FISH, GEP
Plenty of tissue for genomic assays

Formalin-fixed paraffin-embedded biopsies are the "currency" in routine practice
Adaptations have been made in genomic sequencing workflow to allow these biopsies to be used

BIOPSY

An adequate biopsy



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Core Needle

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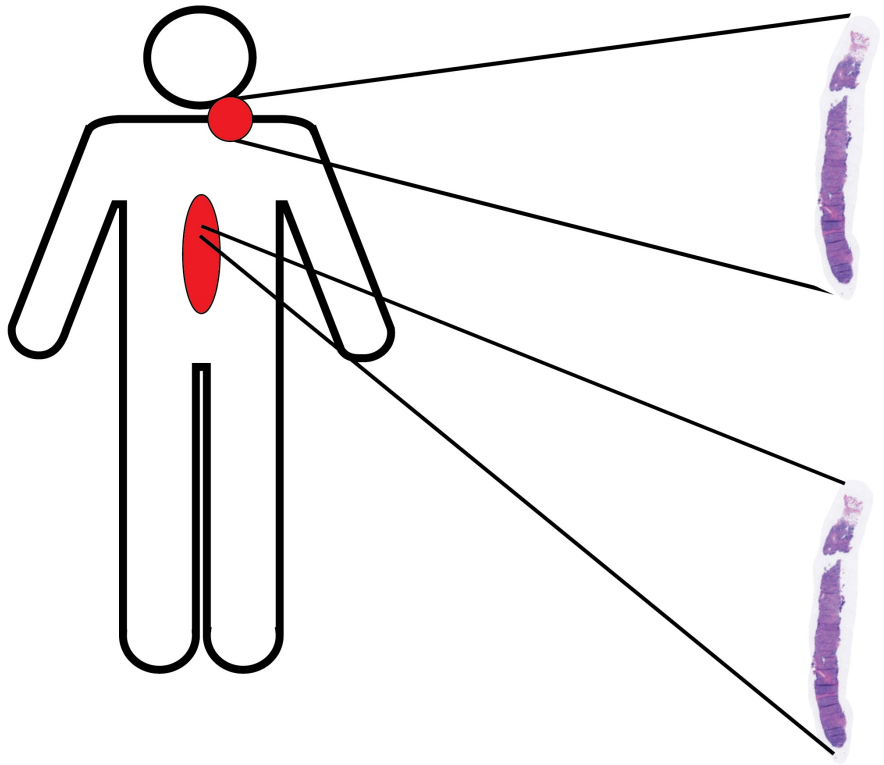
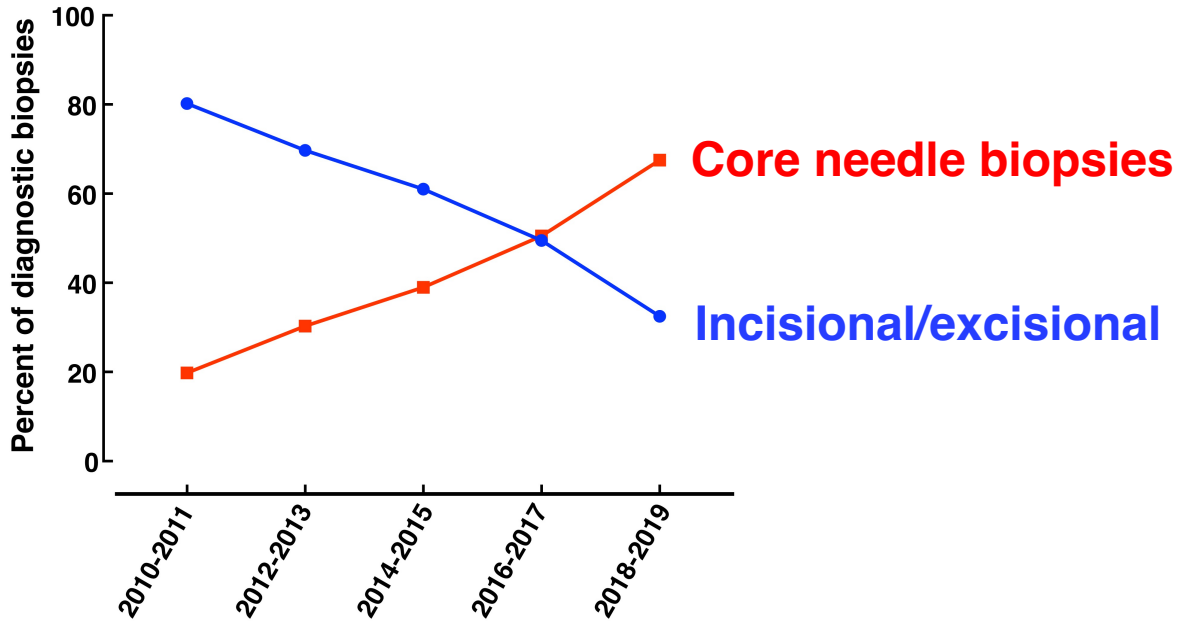
NCCN guidelines (Feb 2023): A core needle biopsy is not optimal but can be used under certain circumstances. In certain circumstances, when a lymph node is not easily accessible……..

BIOPSY

Core needle biopsies – the reality



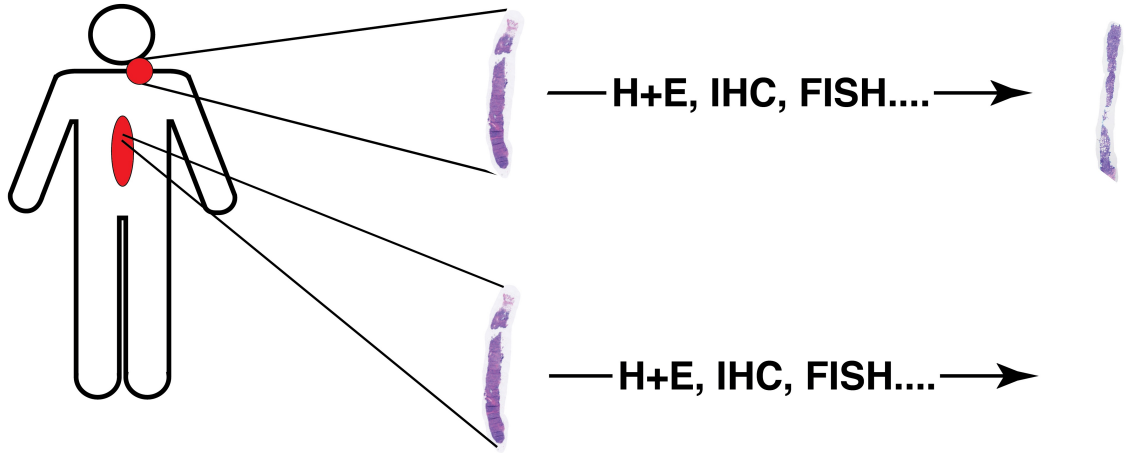


And core needle biopsies are standard practice at the time of relapse when confirmation of the diagnosis is the goal

BC Cancer Pathology data courtesy of Jeff Craig

BIOPSY

Core needle biopsies – the reality



Core needle biopsies are:

- **less likely to provide a definitive diagnosis – 92% vs 98% for excisional biopsies**
- **associated with poor-risk features and inferior outcomes**
- **less likely to provide adequate tissue for molecular analyses**

Syrykh et al Blood 2022

Desai et al Blood Advances 2022

Biopsy adequacy – solutions

- Reverse the trend back towards incisional/ excisional biopsies
- Take dedicated core needle biopsies for genomic studies – placing cores in separate cassettes
- Re-biopsy specifically for genomic studies (fresh)

ASSAY

Genomic assay and platform

- **Assay selection depends on the features being used to assign treatment:**
  - **Mutations in a single gene**
  - **Genetic aberrations organized around pathways**
  - **Broader molecular subgroups (e.g. genetic subgroups)**

This defines target space and –omics requirements

- **Other considerations:**
  - **Tumor content in the specimen**
  - **Importance of clonal structure**

This defines the required depth of coverage

Chapuy et al Nat Med 2018 *Schmitz et al N Engl J Med 2018* *Wright et al Cancer Cell 2020*

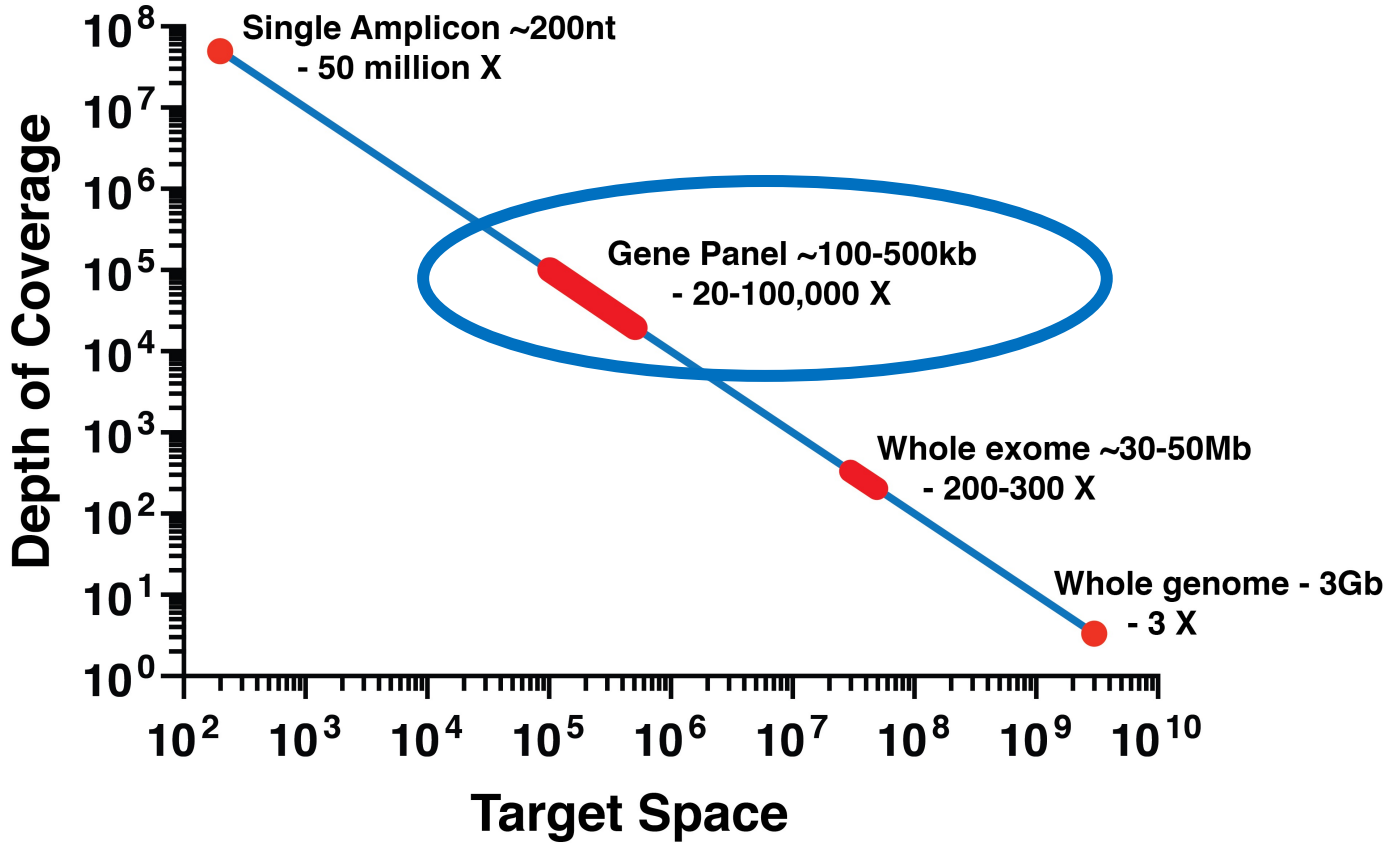
ASSAY

Target space

"Perfect-world" relationship for 10Gb of sequencing

Options:

- Single amplicon
- Gene panels
- Whole exome +/- UTRs
- Whole genome

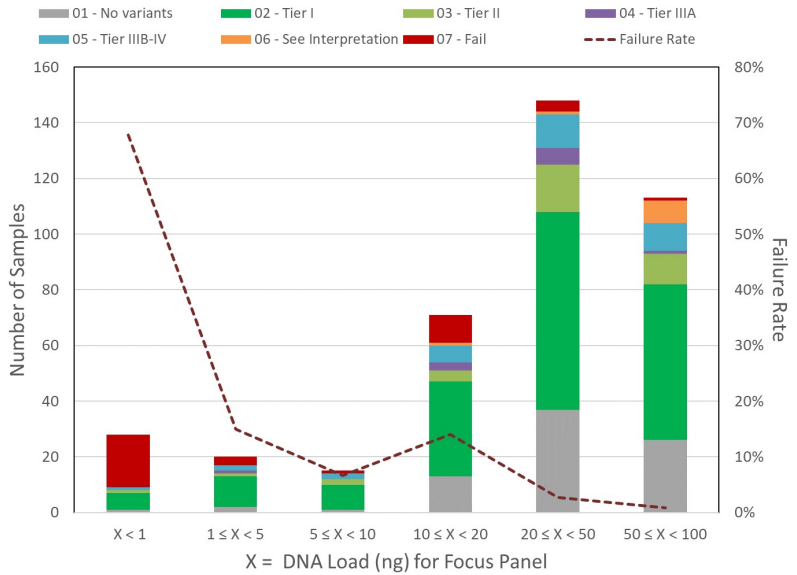


ASSAY

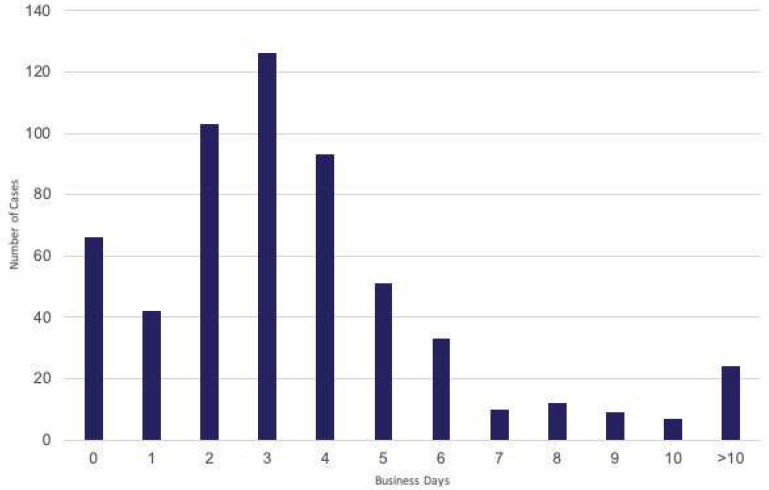
Panel sequencing

- 2 Broad techniques:
  - Amplicon sequencing – specifically amplify the target space
  - Capture sequencing – build a genomic library and then capture

- Advantages of amplicon:
  - Lower inputs needed (100ng or less cf. >200ng)
  - Speed



Turn-around-time in a community setting



BC Cancer CGL Data courtesy of Curtis Hughesman

Sheffield et al Curr Oncol 2022

ASSAY

Panel sequencing

- 2 Broad techniques:
  - Amplicon sequencing – specifically amplify the target space
  - Capture sequencing – build a genomic library and then capture
- Advantages of amplicon:
  - Lower inputs needed (100ng or less cf. >200ng)
  - Speed
- Advantages of capture:
  - Evenness of coverage and less “drop-out” in DNA derived from FFPE
  - Ability to detected moderate size insertions/deletions
  - Relative ease of expanding the target space iteratively
  - Libraries can be used for further characterization
  - Required for a large target space (e.g. whole exome)

ASSAY

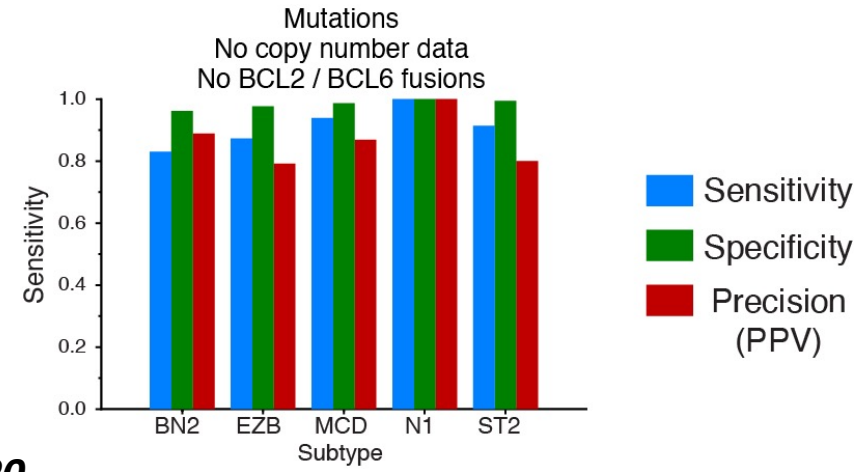
Structural variants

- **Copy number**
  - **Can panel sequencing be optimized for this in FFPE?**
- **Recurrent translocations**
  - **Only a proportion produce fusion genes suitable for capture from RNA**
  - ***MYC* translocations represent a particular issue**

ASSAY

Assigning genetic subgroups

- A method to assign tumours on a biopsy-by-biopsy basis is required
- The probabilistic model that does this currently leaves 37% of tumours unassigned – “Other”
- What are the minimal genetic features to assign subgroups?



Note:

1. **Copy number is required for A53**
2. **What happens to the proportion of “Other” as the features are reduced?**

Wright et al Cancer Cell 2020

Bioinformatics/reporting

- **Bioinformatics**
  - **Many different algorithms are being used to call genetic aberrations**
  - **No universally accepted standard**
  - **Assay and bioinformatic approaches to C➔T FFPE artifacts**
  - **Is germline needed?**

- **Reporting**
  - **Different thresholds for variant allelic frequency (VAFs) – 5% vs 1%**
  - **Reporting of variants of uncertain significance (VUSs)**

Centralized vs distributed testing

- Centralized testing
  - Standardized procedures, assay, bioinformatics and reporting
- Distributed testing
  - Potentially reduced turn-around-time
  - More likely to reflect what will occur in routine patient care
  - Requires harmonization and standardization

THERAPY SELECTION

Treatment assignment

- **Molecular Tumor Boards:**
  - **Flexibility**
  - **Allows consideration of factors beyond the genomic results**
  - **Adds time to the process**

MOSCATO 01 Trial

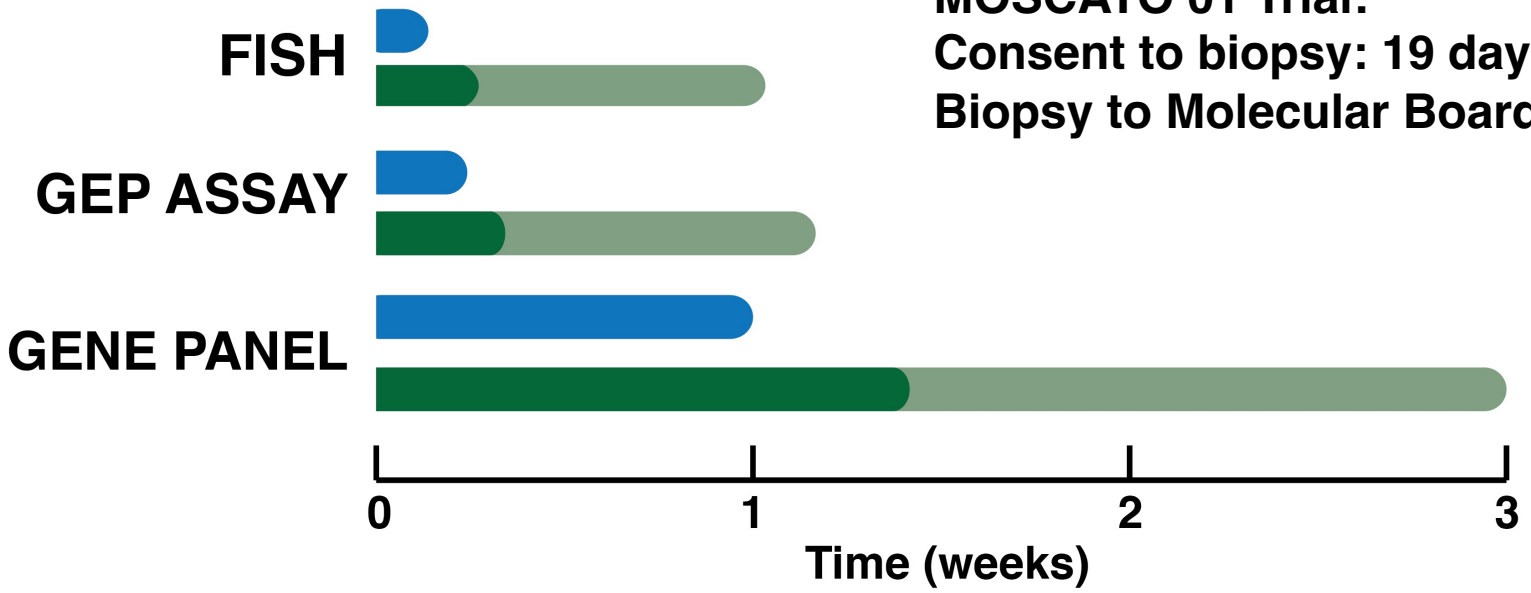
- **Algorithms:**
  - **Set rules established ahead of time**
  - **Reproducibility of assignments**
  - **Rapid turn-around-time**

NCI-MATCH

Massard et al Cancer Discov 2017

THERAPY SELECTION

Streamlining – reducing delays



MOSCATO 01 Trial:
Consent to biopsy: 19 days (IQR 6-33)
Biopsy to Molecular Board: 21 days (IQR 14-27)

While we obsess over the theoretic assay turn-around-time, much of the delay is related to assay batching, time to decision to do the testing, obtaining the materials and shipping

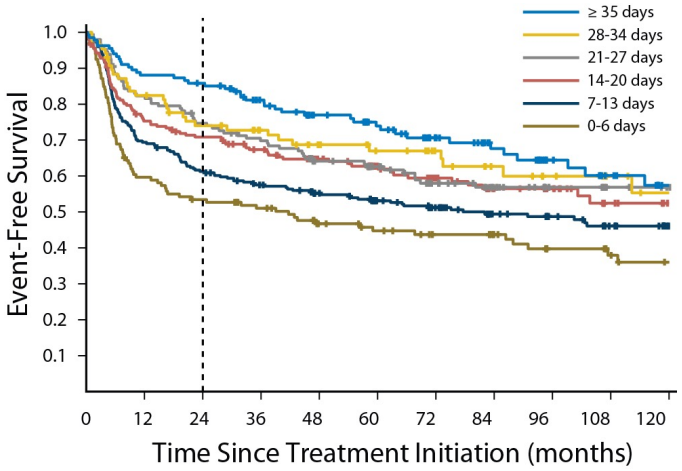
Solution: Integrate sequencing into routine pathology practice

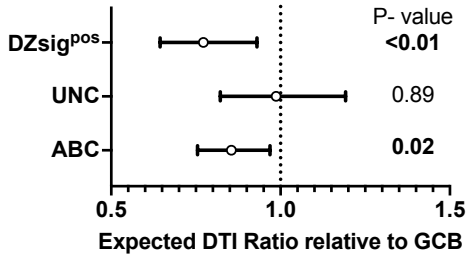
Massard et al Cancer Discov 2017

THERAPY SELECTION

Trial design

- In aggressive lymphoma many patients need rapid treatment
- Patients that are treated rapidly have inferior outcomes
- Generalizability requires enrolment of patients that need immediate treatment
- These patients likely have the most to gain





Solution: Build bridging therapy into the trial design

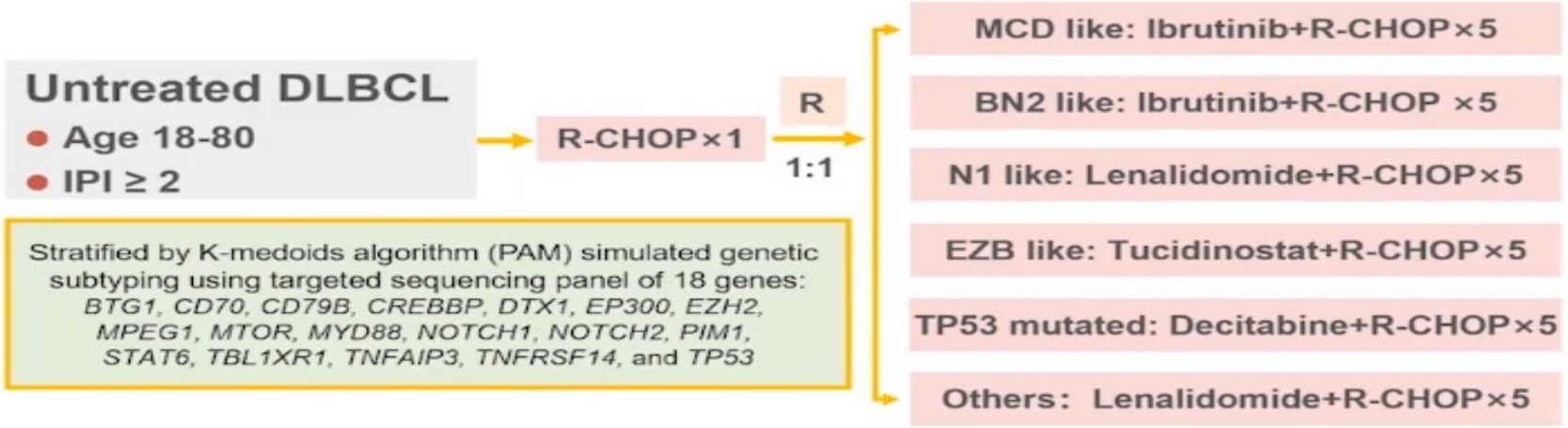
Maurer et al J Clin Oncol 2018

Alduaij, Collinge et al Blood 2022

Guidance-01: Randomized Phase 2 Trial of Genetic Subtype Guided Immunochemotherapy

Study Design (NCT04025593)

- The study started from **July, 2019**.
- All patients were treated with ONE cycle of standard R-CHOP immediately at diagnosis.
- Patients were randomly assigned 1:1 and stratified by genetic subtype.
- Using targeted sequencing and FISH for BCL2, MYC translocation and BCL6 fusion to classify patients into six genetic subtypes MCD like, BN2 like, N1 like, EZB like, according to **NEJM classification (2018)**, *TP53* mutation, and others.



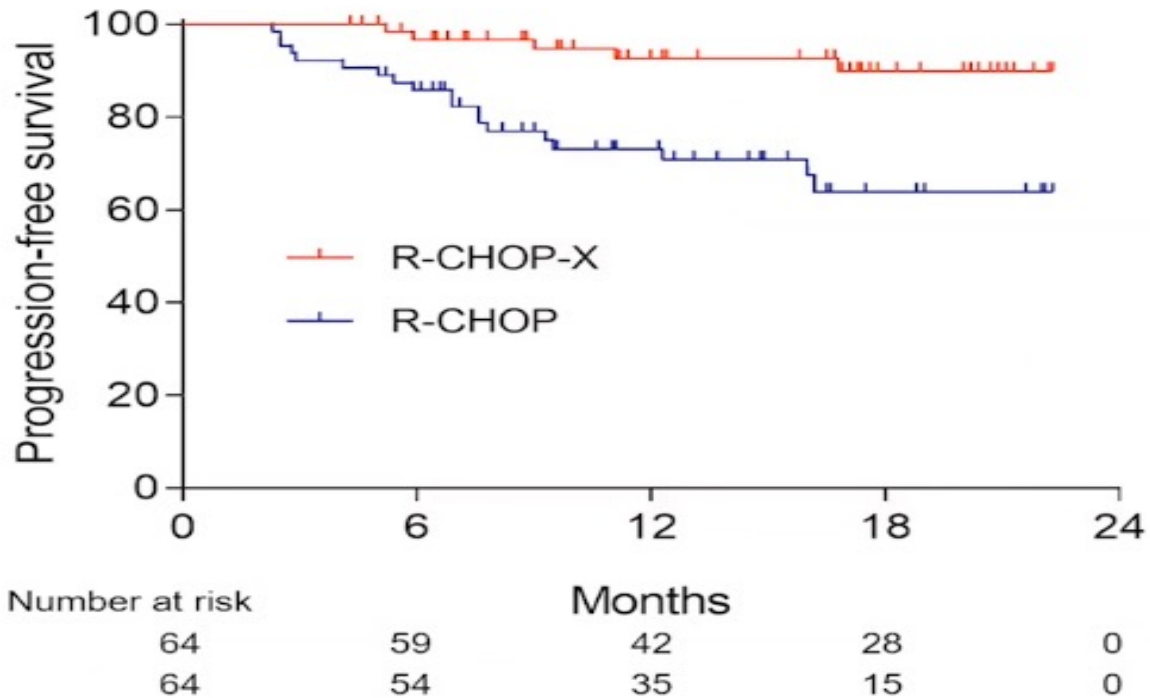
Ibrutinib[1]	420mg po qd
Lenalidomide[2]	25mg d1-10 po
Tucidinostat[3]	20mg d1, 4, 8, 11 po
Decitabine[4]	10 mg/m² d1-5
R-CHOP	Standard dose

G-CSF prophylaxis was given from the second cycle of chemotherapy if grade ≥ 3 neutropenia was present in the first cycle.

1. *Younes et al., J Clin Oncol* 2019. 2. *Nowakowski et al., J Clin Oncol* 2021. 3. *Zhang et al., Clin Epigenet* 2020. 4. *Zhang et al.*, ICML 2019 abstract (NCT02951728)

Zhang et al ICML 2021

Secondary Endpoint: PFS



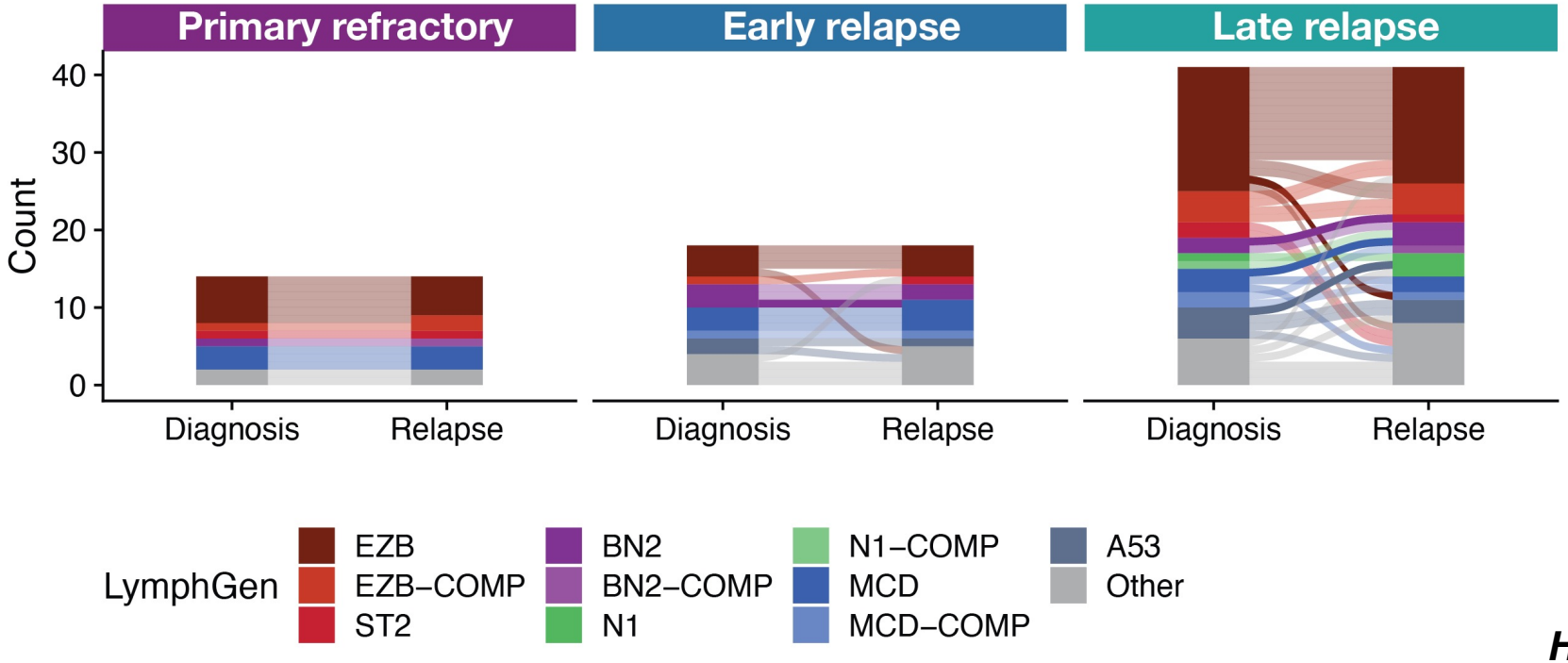
Median follow-up 16.1 months

	R-CHOP-X	R-CHOP
1-year PFS (95%CI)	93% (81%-97%)	73% (60%-83%)

Zhang et al ICML 2021

Do you need to sequence at relapse?

- **LymphGen subgroups are consistent between diagnosis and relapse, even in "late relapses" (>2 years from diagnosis to relapse)**

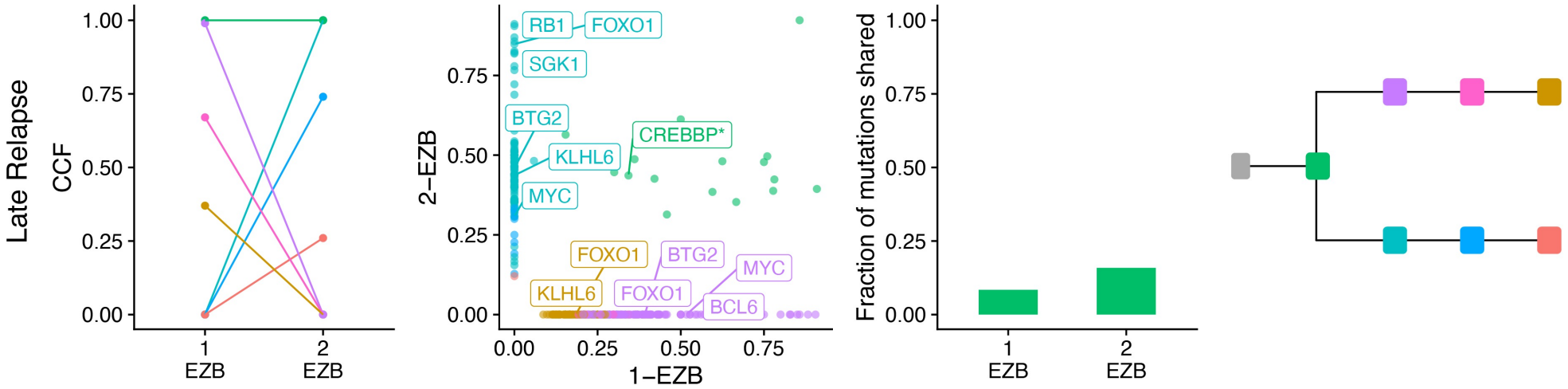


Whole genome/whole exome with germline sequencing from diagnostic and relapse biopsies from 73 patients

Hilton et al ASH Annual Meeting 2022

Do you need to sequence at relapse?

- **LymphGen subgroups are consistent between diagnosis and relapse, even in "late relapses" (>2 years from diagnosis to relapse)**
- **However, late relapses actually represent new DLBCL emerging from common precursor cells and harbour different mutations**



Hilton et al ASH Annual Meeting 2022

Do you need to sequence at relapse?

- LymphGen subgroups are consistent between diagnosis and relapse, even in “late relapses” (>2 years from diagnosis to relapse)
- However, late relapses actually represent new DLBCL emerging from common precursor cells and harbour different mutations
- So, if you are targeting a specific mutation or pathway, sequencing the relapse is recommended

de Leval Blood 2022

Hilton et al ASH Annual Meeting 2022

Summary

- **Matching treatment to tumour biology holds the promise of improved patient outcomes**
- **Each component of the process from biopsy to treatment selection represents distinct practical challenges**
- **In order for this approach to benefit patients the process needs to be streamlined and integrated into routine diagnostic practice**