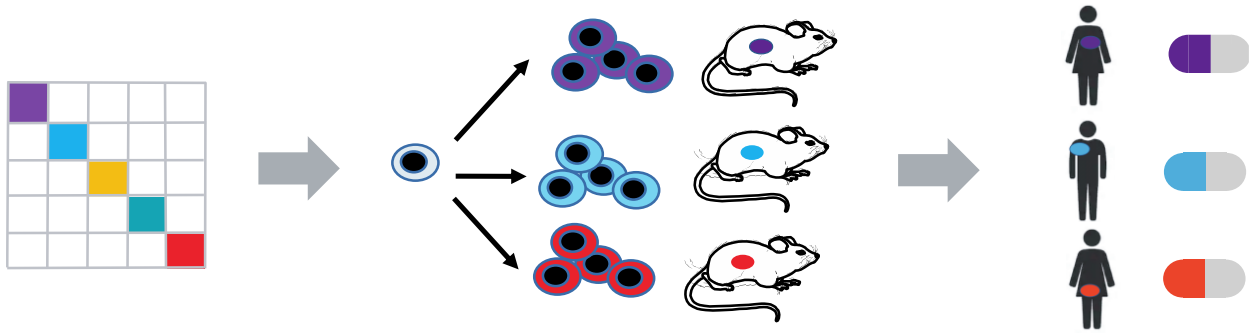
Bologna Aggressive Lymphoma Workshop

Precision Medicine in DLBCL – Molecular Predictors of Response



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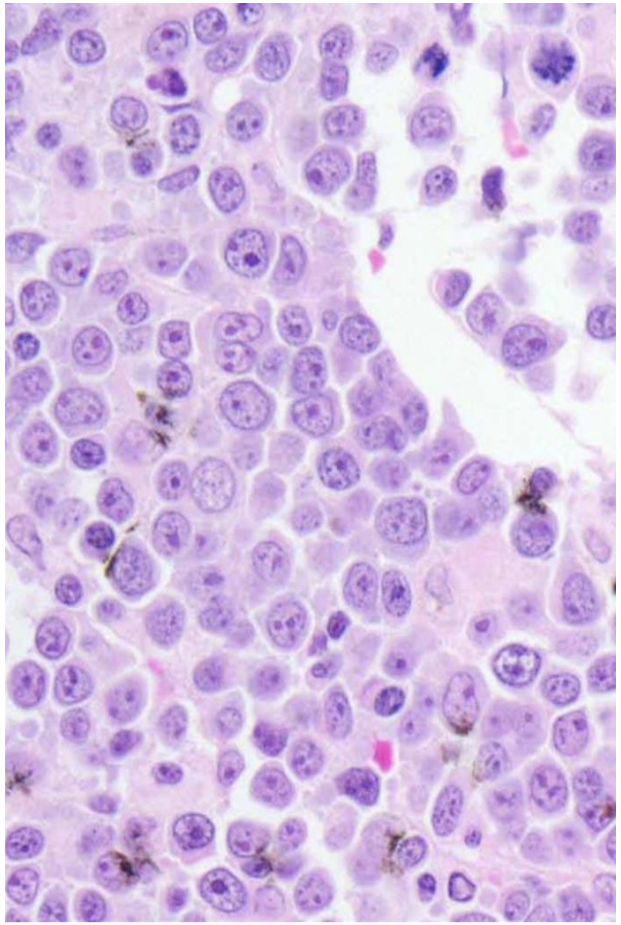
May 8,223

Disclosures

- I have the following financial relationships to disclose:
  - *Research support from* Gilead Sciences:
    Gilead Oncology Award Winner 2021 (with S. Dietrich)
    Gilead Oncology Award Winner 2018
  - *Honoraria for,*
    invited talks BMS, Astra Zeneca, Gilead, Roche, Sandoz, Incyte, Abbvie, Sobi
    advisory boards Regeneron, BMS, Roche, ADC, Incyte, Abbvie, Sobi
  - *Patents* I hold several patents on molecular subtyping of large B-cell lymphoma

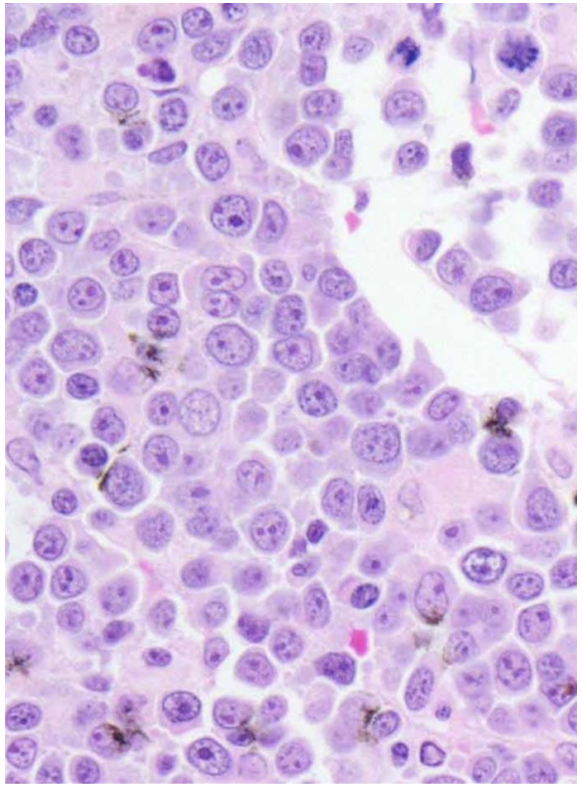
Diffuse Large B-cell Lymphoma (DLBCL)

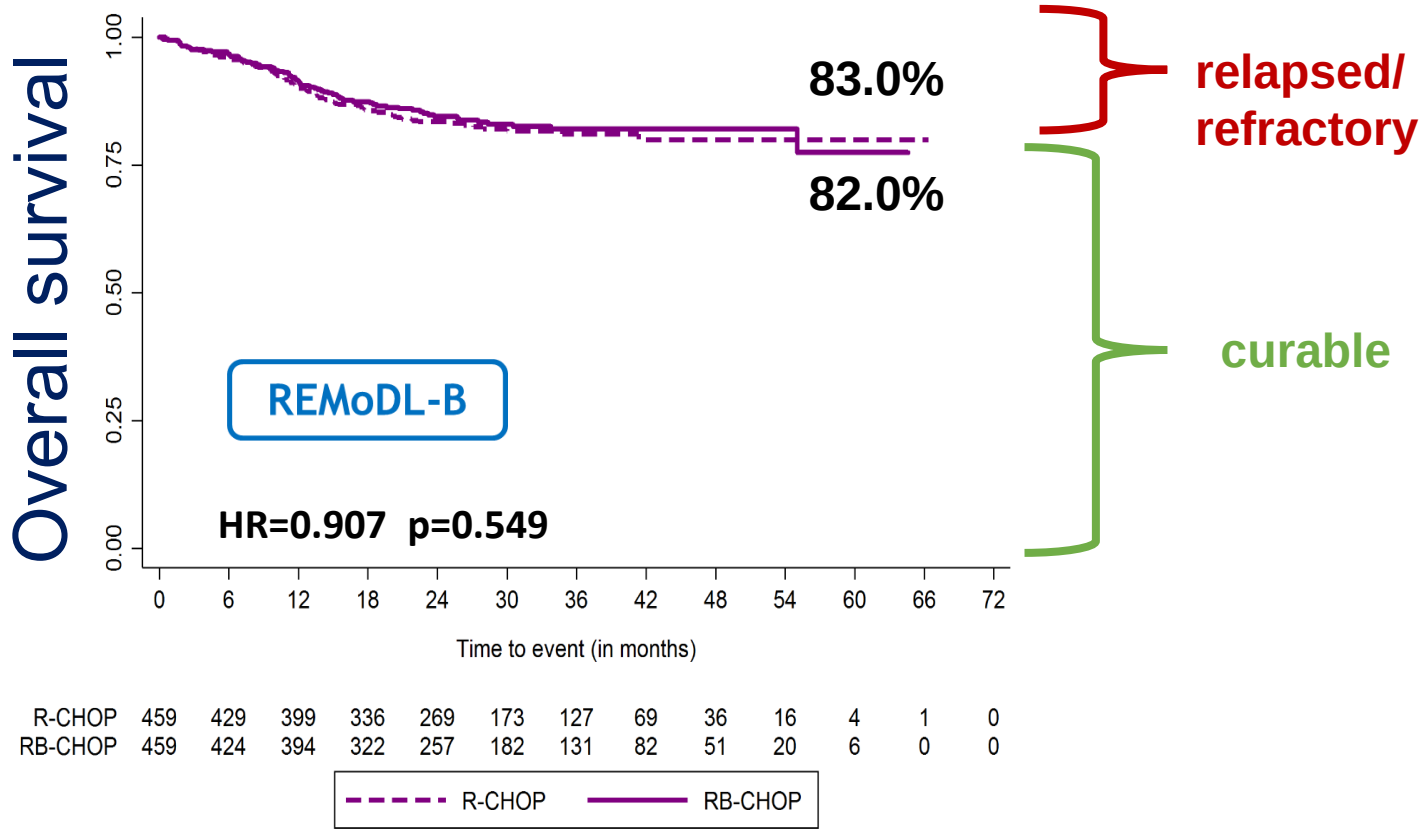


- Most common aggressive Non-Hodgkin lymphoma in adults.
- Arises from antigen-exposed germinal center B-cells.
- Separated from other LBCL such as high-grade B-cell lymphoma, PMBL and PCNSL.
- Molecular heterogenous disease with recognized transcriptionally subtypes with distinct functional characteristics.
- Genetically-defined DLBCL subtypes recently discovered.

➔Despite a more granular picture on the molecular insights of DLBCL have the perspectives of patients over the last 20 years only marginally changed.

DLBCLc
One disease, one treatment?



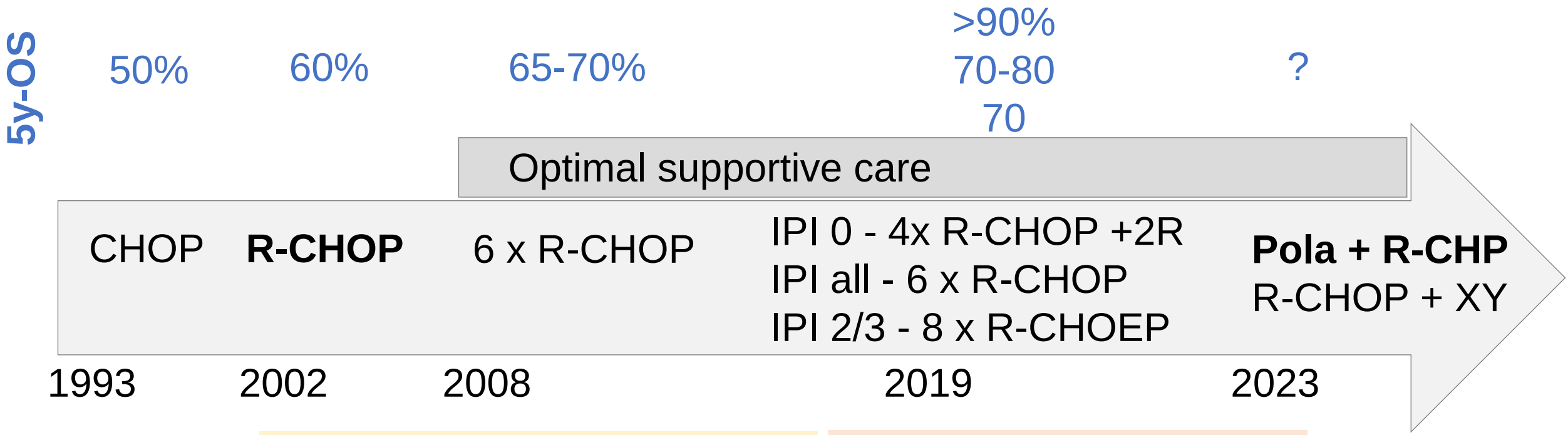


➔ R-CHOP-like treatments is the established standard since decades.

https://imagebank.hematology.org/image/1811

Davies ICML 2017

Empirical Optimization of R-CHOP



"failed"

Longer treatment (8 vs 6)
Dose-density (14 vs. 21)
Higher doses (Mega)
Early Transplant
Infusional applications
New CD20 antibodies

R-CHOP + X 'all comer' designs

X = Bortezomib
X = Lenalidomide
X = Ibrutinib
X = other

Strategies Towards Precision Medicine (in Lymphoma)

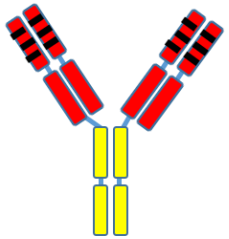
Molecular agnostic

“All comer” Studies
oriented towards surface epitopes

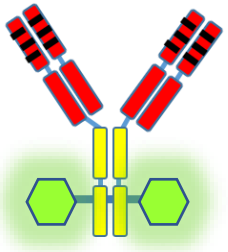
Precision Medicine in Lymphoma - New Bullets on the ~~"Horizon"~~ available

Targeting Surface Epitopes

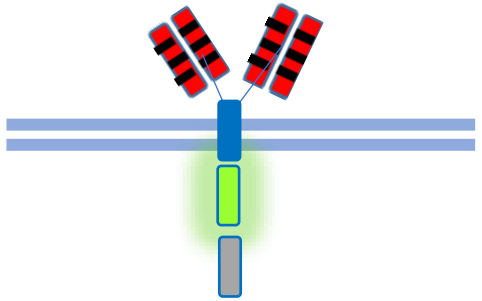
Chemo



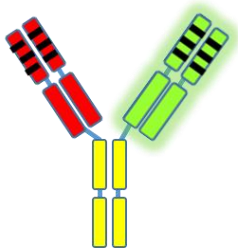
Antibodies



Antibody Drug Conjugats



CAR T-Cells & friends

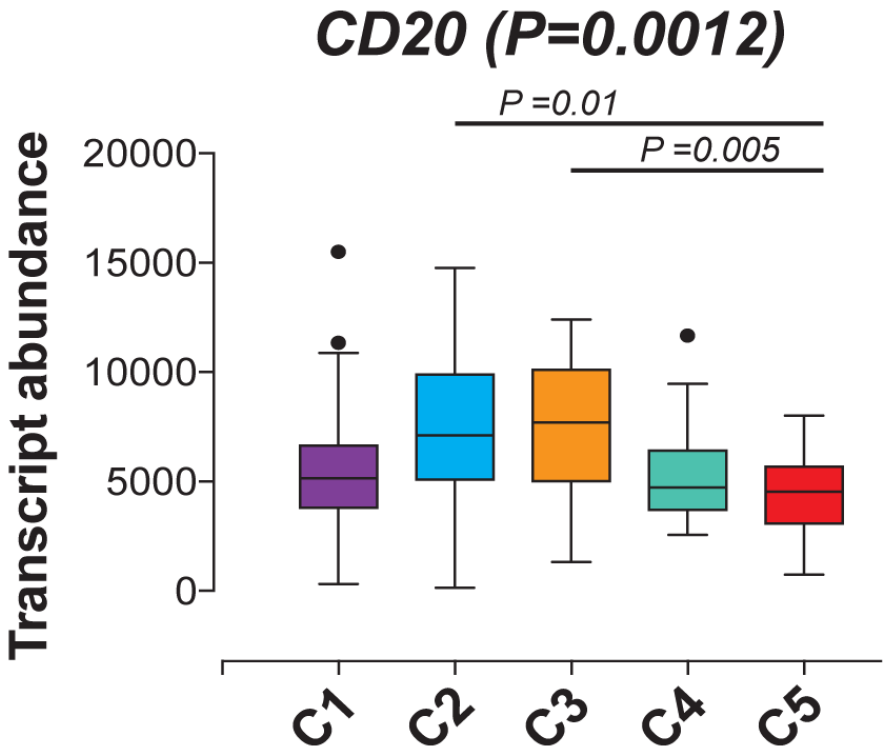


bispecific Antibodies

Empirical Strategy

- Combine as single agent or smart combinations in all comer trials
- If needed (i.e. primary end point is failed) look for biomarkers and understand molecular heterogeneity

Heterogenous Abundance of CD20 in Genetic C1-C5 DLBCL Subtypes

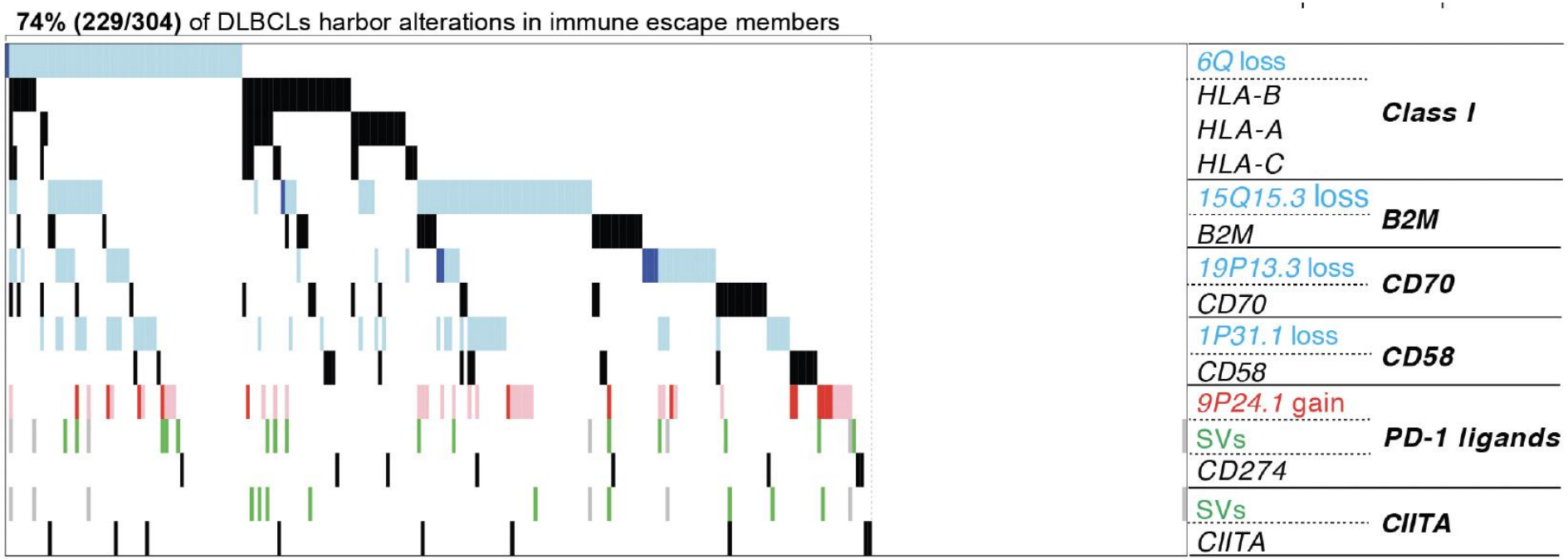


- CD20 transcript abundance is significantly different in genetically defined subtypes

➔ Highlights that epitope density varies for so called “agnostic” therapies

Chapuy Nat Med; 2018; 24(5):679-690

Frequent Immune Escape Pathways in DLBCL



➔ 2/3 of DLBCL patients have genetic alterations in a potent immune escape pathways

Chapuy et al Nat Med 2018

Strategies Towards Precision Medicine (in Lymphoma)

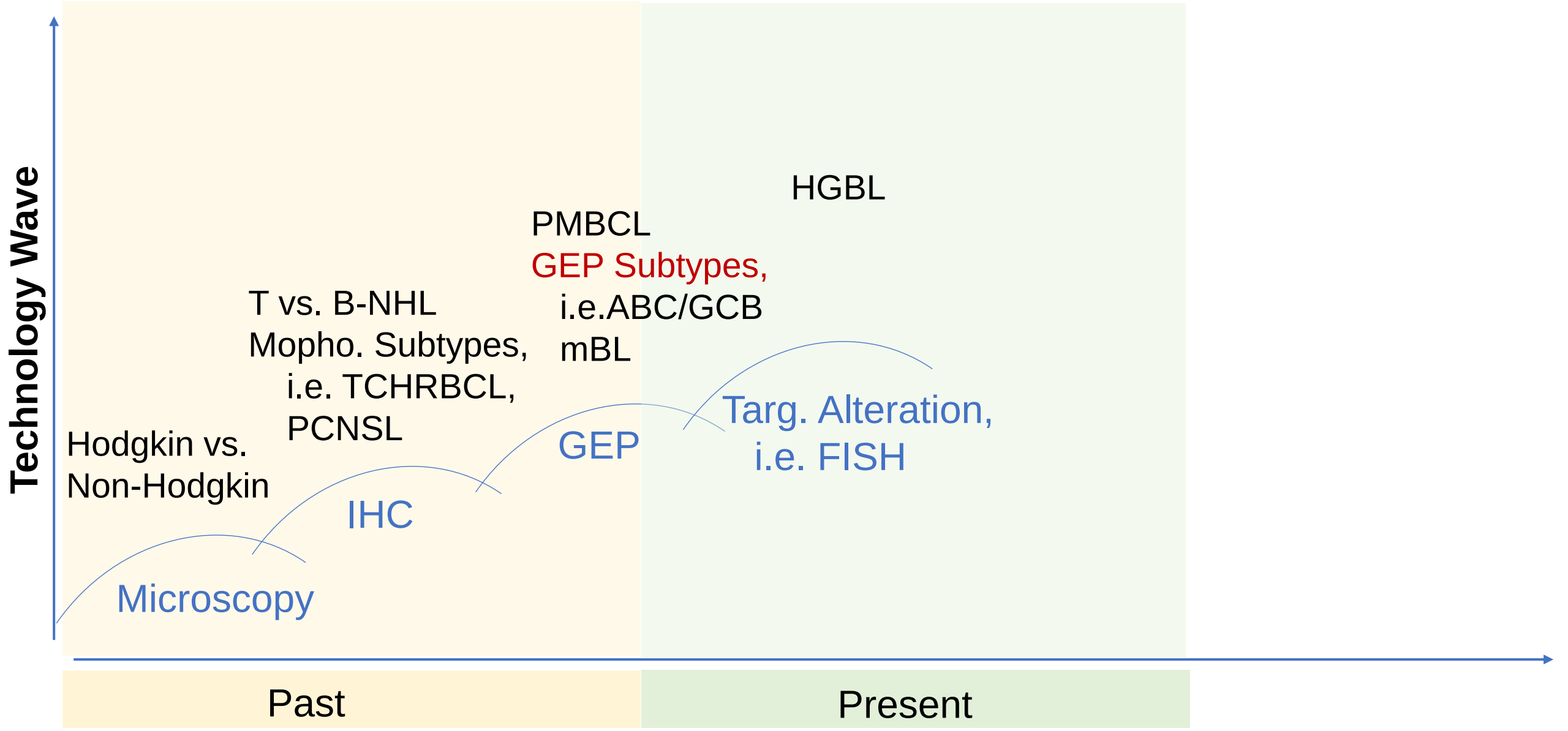
Molecular agnostic

“All comer” Studies
oriented towards surface epitopes

Molecular affine

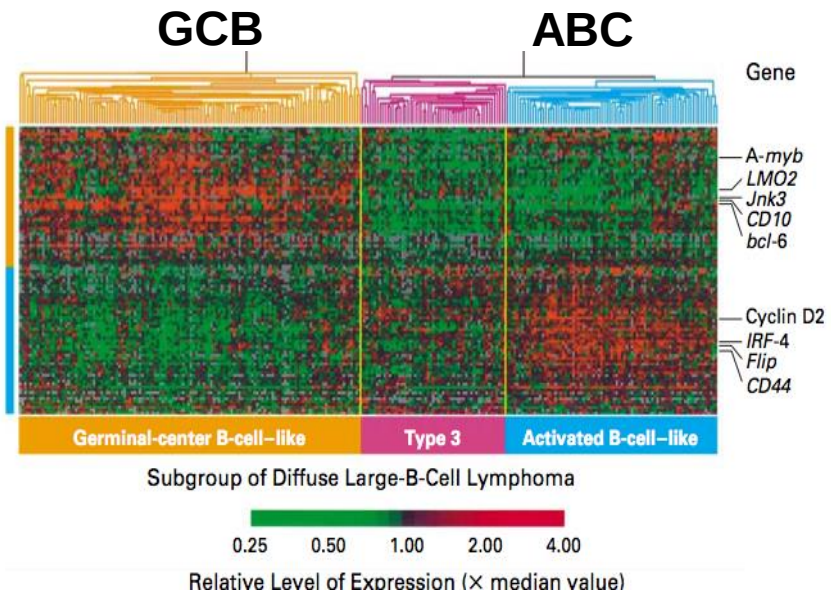
Understanding Molecular Heterogeneity
&
Targeting Actionable Alterations

Evolving Molecular Classification with Technology



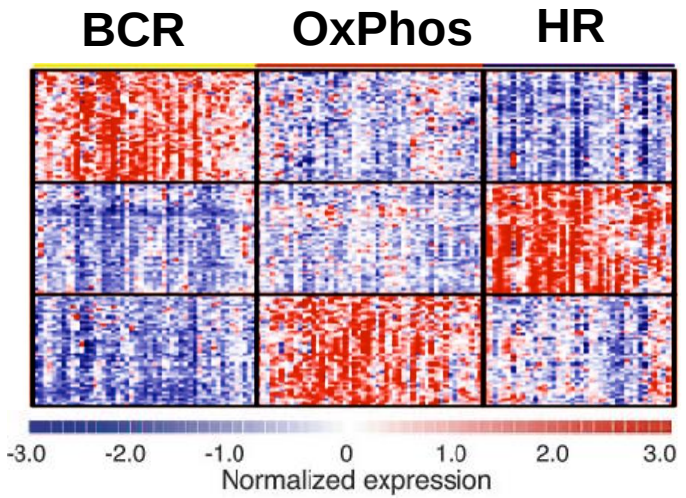
Transcriptional Heterogeneity in DLBCL

Cell of origin



Alizadeh et al, Nature 2000
Rosenwald et al, NEJM 2002
Lenz et al NEJM 2008
Lenz and Staudt NEJM 2010

Consensus Clusters

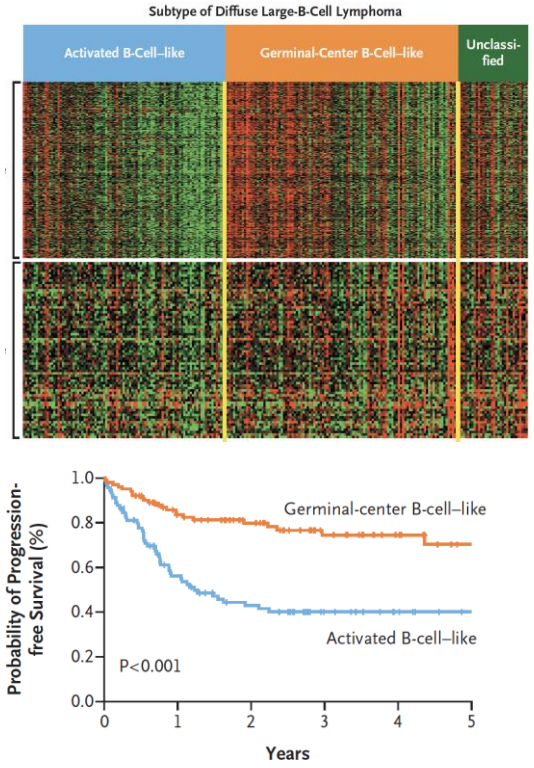


Monti et al, Blood 2005
Chen et al, Cancer Cell 2012
Caro et al, Cancer Cell 2013

- Transcriptionally defined disease subtypes highlight specific aspects of DLBCL biology, suggest cancer cell dependencies and identify rational therapeutic targets.

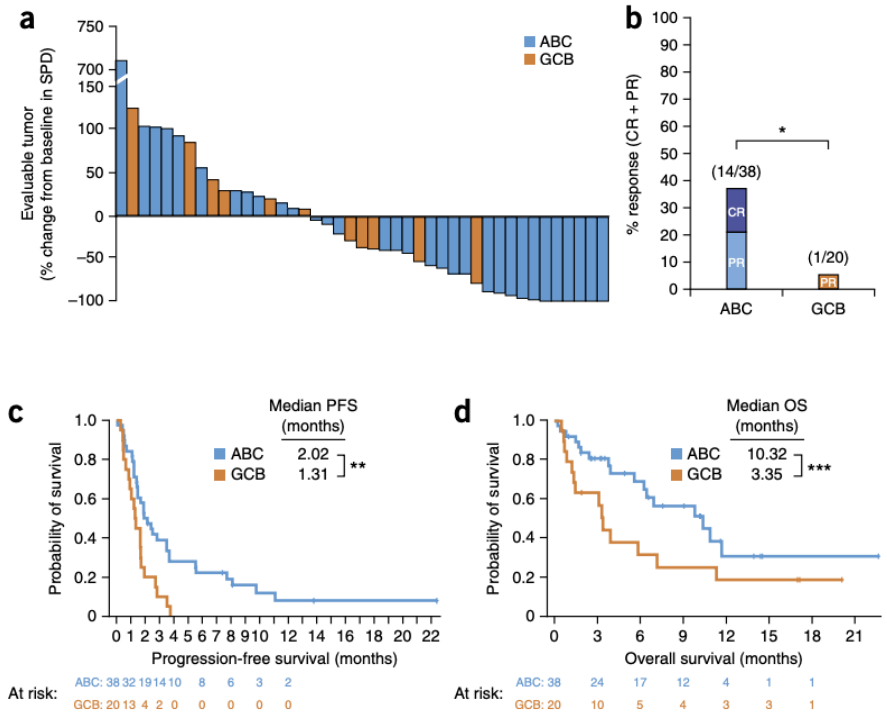
Targeting ABC-type DLBCL

Transcriptional Heterogeneity of DLBCL



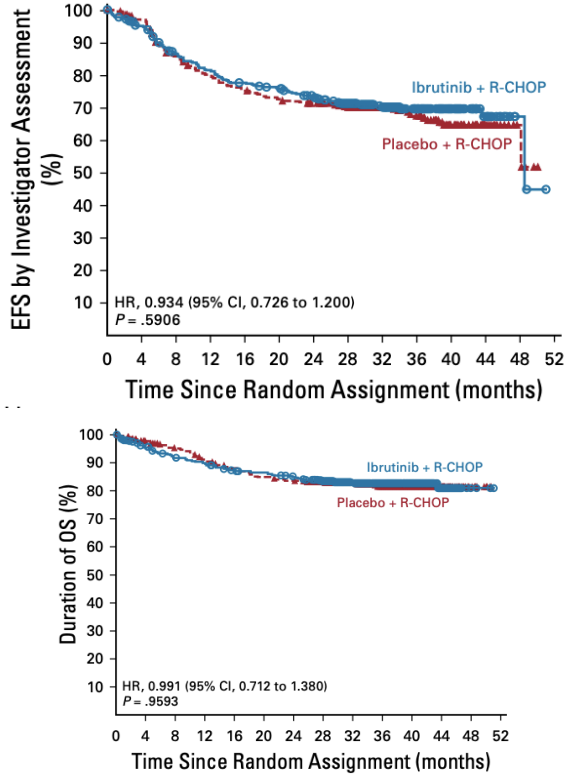
Lenz et al. *N Engl J Med* 2008;359:2313-23

Vulnerability of ABC to BTK Inhibition



Wilson et al. *Nat Med.* 2015; 21, 922–926.

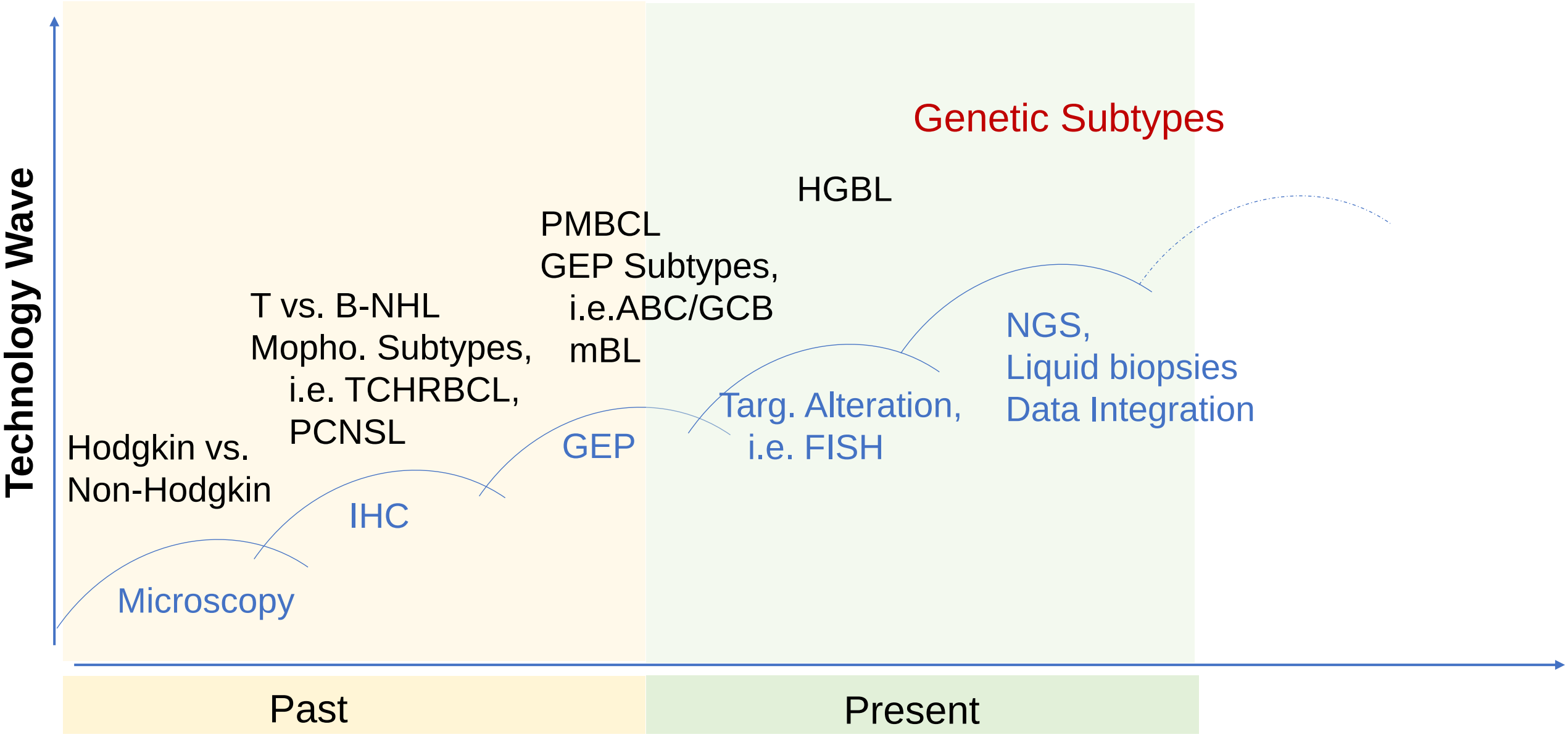
Phase III Trial Failed End Point



Younes et al. *JCO.* 2019; 20;37(15):1285-1295

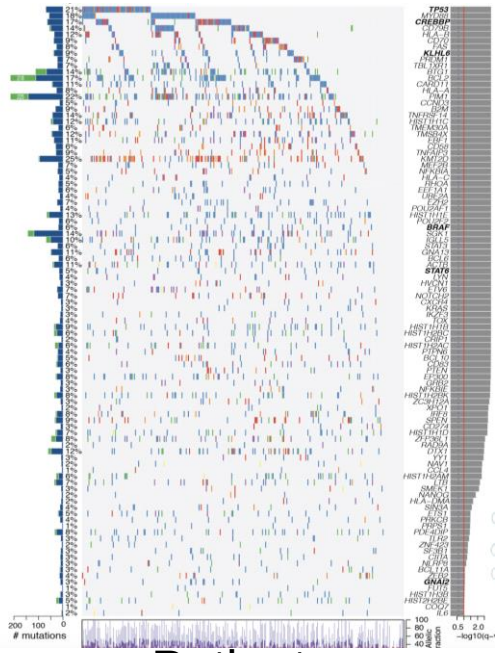
➔ Suggested that there is additional molecular heterogeneity

Evolving Molecular Classification with Technology

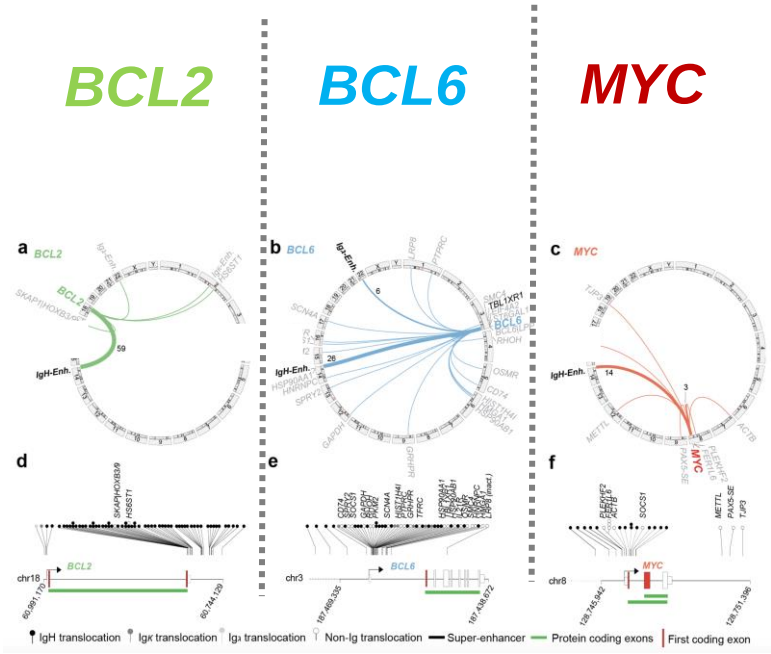


Comprehensive Genomic Analysis of Primary DLBCL

Mutations



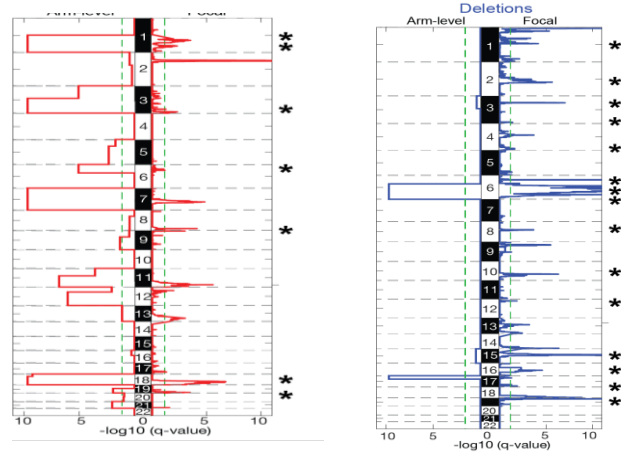
SVs



SCNAs

Amplifications **Deletions**

Arm-level Focal Arm-level Focal



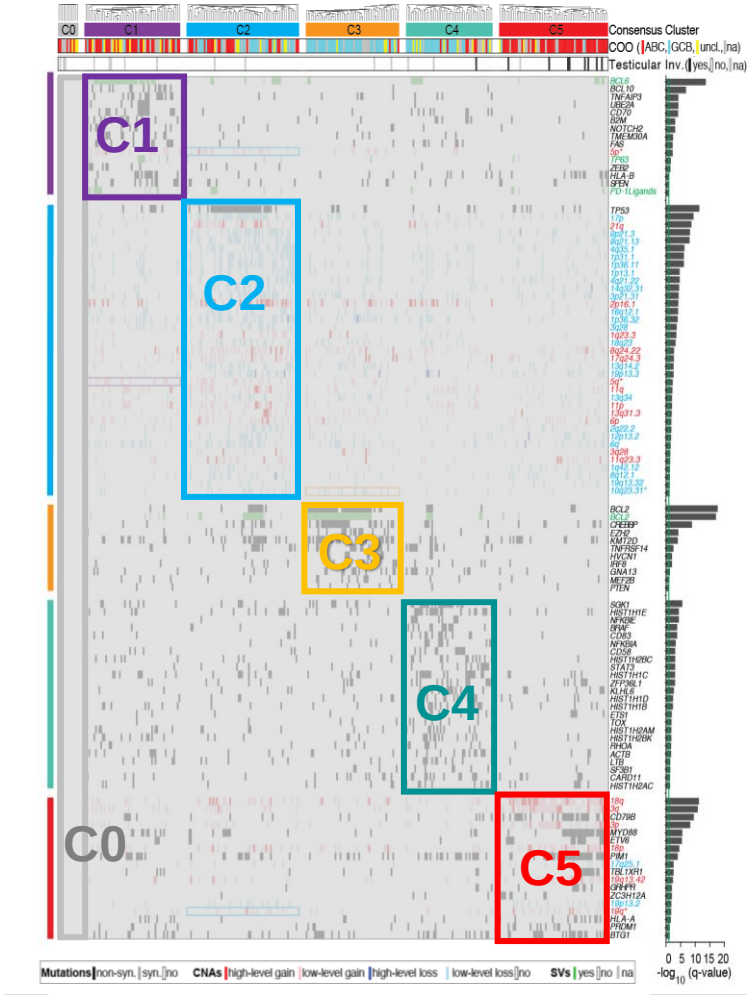
* Significantly mutated genes within SCNAs

- Integration of recurrent mutations, somatic copy number alterations (SCNAs) and structural variants (SVs) in newly diagnosed DLBCLs.
- Median # of genetic driver alterations is 17 (1-48)

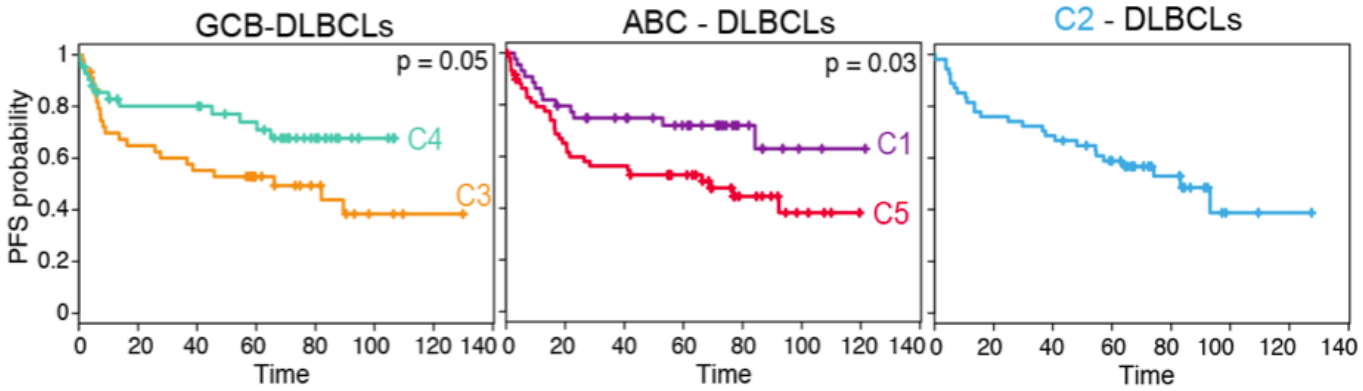
GOAL: Define DLBCL genetic substructure

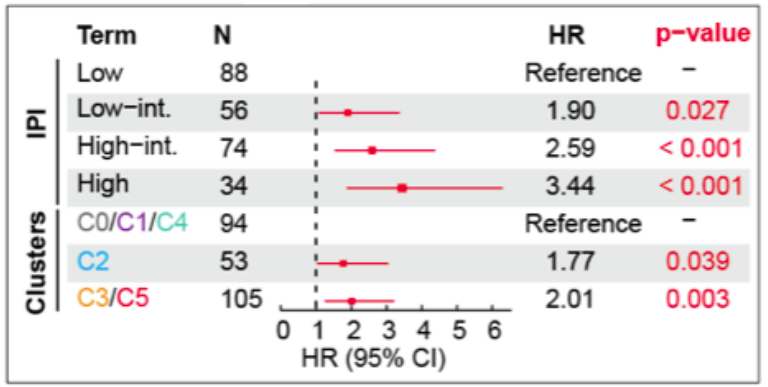
Genetic Signatures Predictive for Outcome Independent of the IPI

Genetically-distinct DLBCLs



Predictive for Outcome

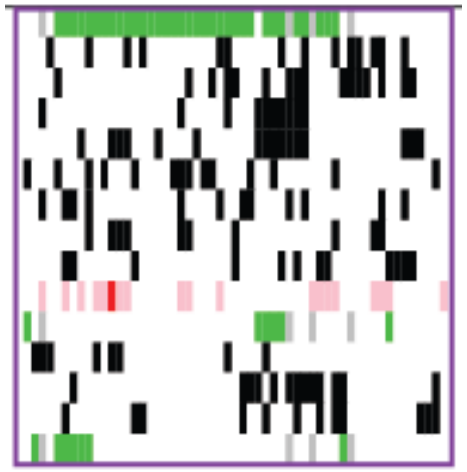


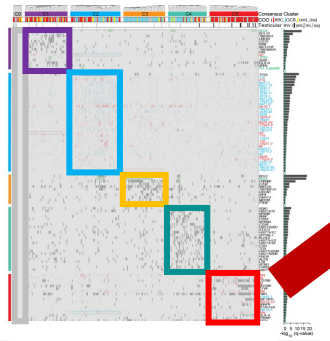


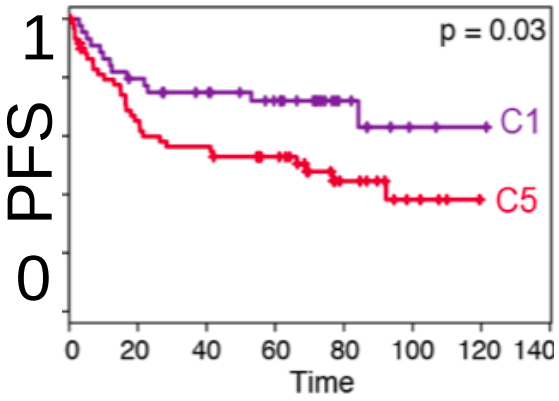
Chapuy, Stewart, Dunford et al. *Nat Med;* 2018; 24(5):679-690

Genetically Distinct ABC-enriched DLBCLs

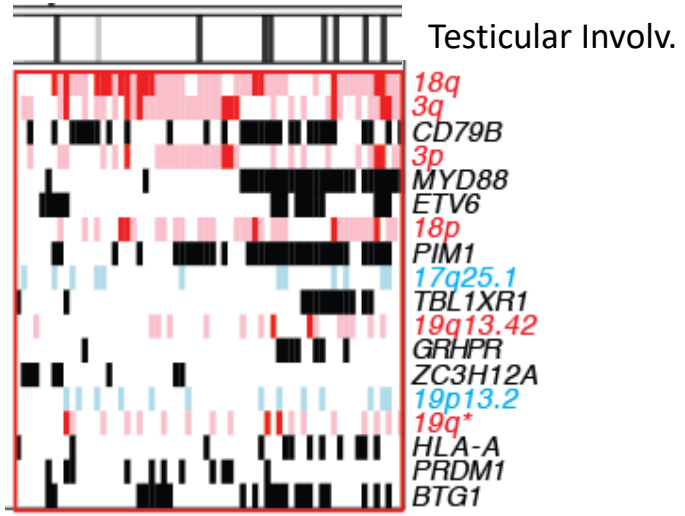
C1 DLBCLs







C5 DLBCLs



- Mutations as previously described in marginal zone lymphoma **(MZL)**[1-4]
- *BCL6* SVs associated with **transformed MZL**[5]
- **Favorable** outcomes

➔ **20%** of DLBCLs occultly transformed **MZL ?**

- 18q/*BCL2* gain with concurrent mutations in *MYD88*^{L265P}/*CD79B*
- Resembled genetic sign. of **PCNSL and PTL**[6] and **other extranodal lymphoma**[7]
- **8/9 DLBCL with testicular involvement**
- **Unfavorable** outcome

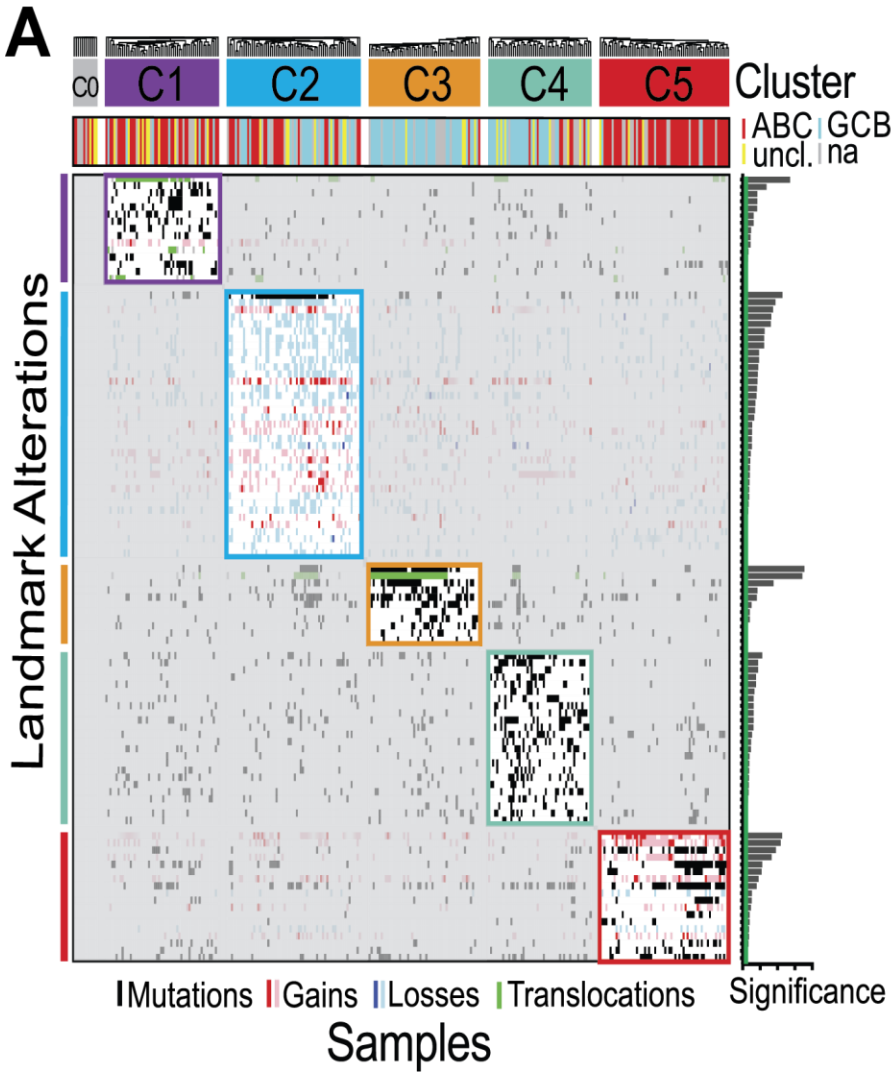
➔ Coordinate genetic signature associated with extranodal tropism.

[1] *Zhang et al., Nat. Gen 1999*
[2] *Rossi et al., JEM 2012*
[3] *Kiel et al., JEM 2012*
[4] *Spina et al., Blood 2016*
[5] *Flossbach et al., Int J Cancer 2011*
[6] *Chapuy, Roemer et al., Blood 2016*
[7] *Wright et al Cancer Cell 2020*

Chapuy, Stewart, Dunford et al. Nat Med; 2018
Wienand and Chapuy. *Hem Oncol* 2021

Genetically-distinct DLBCLs and their Associated Features

A

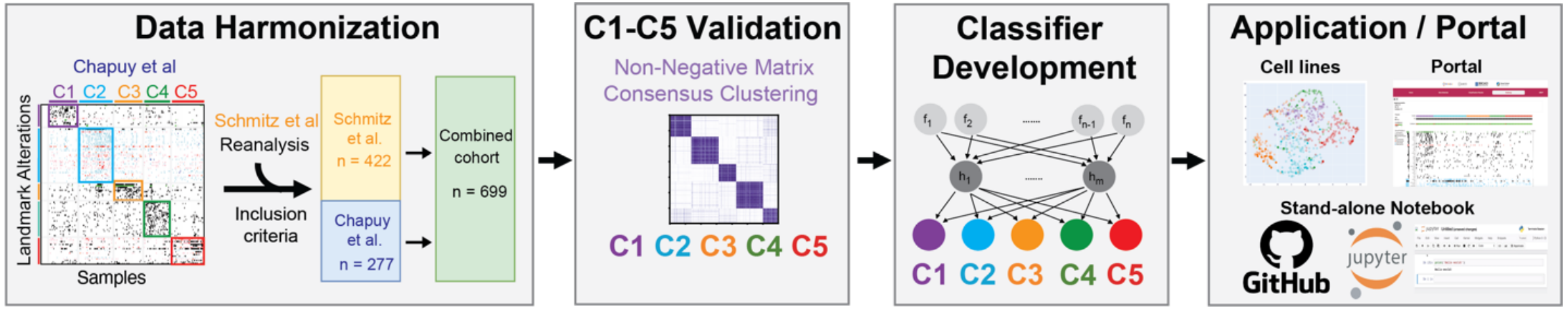


B

Genetic Subtype *DFCI*	Genetic Subtype *NCI*	Key Features	Transcriptional Subtype	Prognosis
C1	BN2	*BCL6* and NOTCH2- and NF-κB and immune escape pathway alterations; occulty transfomed MZL	ABC	Favorable
C2	(A53 / TP53)	Biallelic inactivation of *TP53*, 9p21.3/*CDKN2A;* genomic instablity	ABC/GCB independent	Steady rate of progression
C3	EZB	*BCL2* SVs, inactivating *PTEN* alterations and alterations of epigenetic enzymes	GCB	Unfavorable
C4	ST2	BCR/PI3K-, JAK/STAT-, RAS-pathway and histone alterations	GCB	Favorable
C5	MCD	*BCL2* copy gain, activating *MYD88* and *CD79B* mutations; extranodal tropism	ABC	Unfavorable

Wienand and Chapuy, ICML 2021

Molecular Classificator for DLB*class*



a

Train/Validation Set

DLB*class*	C1	C2	C3	C4	C5
C1	107	2	1	1	3
C2	1	117	1	5	3
C3	2	4	82	2	5
C4	3	2	2	64	4
C5	3	2	3	1	130
Accuracy	**91% (500/550)**				

NMF lables / All cases

Conf. > 0.7	C1	C2	C3	C4	C5
C1	85	1	0	0	1
C2	0	97	1	0	1
C3	1	2	66	0	1
C4	2	0	2	44	1
C5	0	0	0	0	109
Accuracy	**97% (401/414)**				

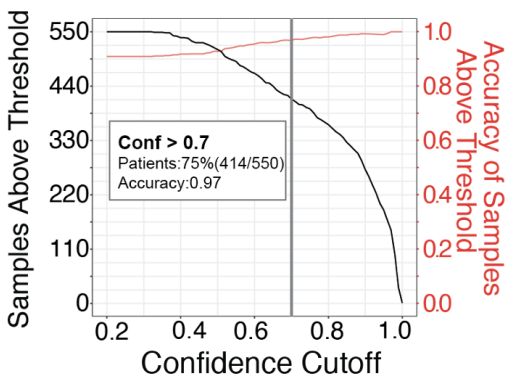
Test Set

	C1	C2	C3	C4	C5
C1	21	0	1	4	0
C2	1	39	0	2	2
C3	0	0	21	1	0
C4	1	0	1	19	1
C5	0	2	1	0	32
Accuracy	**89% (132/149)**				

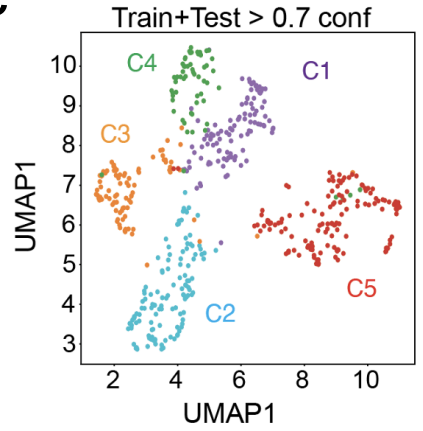
Conf > 0.7

	C1	C2	C3	C4	C5
C1	18	0	0	2	0
C2	0	26	0	0	0
C3	0	0	21	0	0
C4	0	0	0	16	0
C5	0	0	0	0	27
Accuracy	**98% (108/110)**				

b



c



Properties

- Robust classification of single cases
- Output: C1-C5, probabilistic
- "easy-to-use" online tool

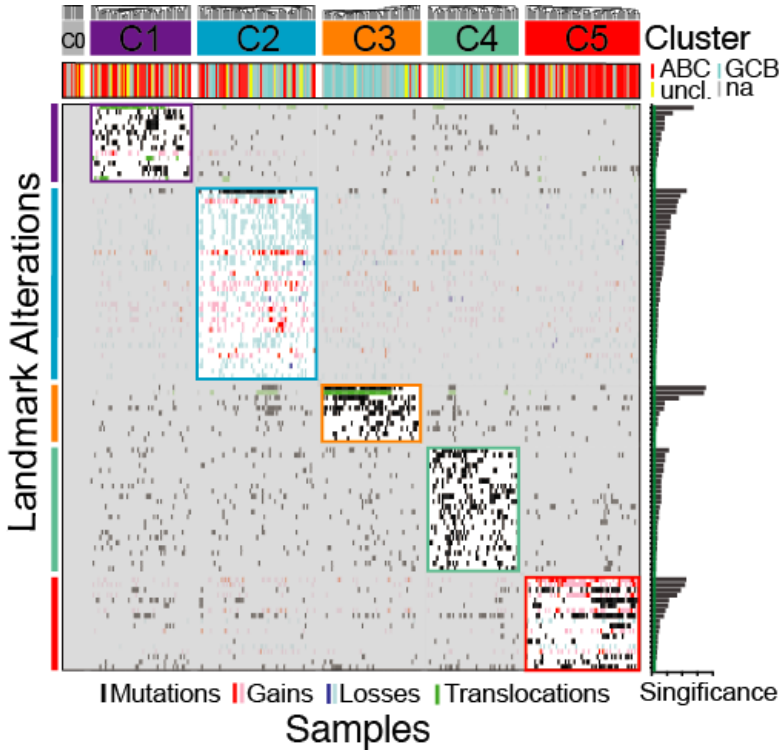
DLBclass

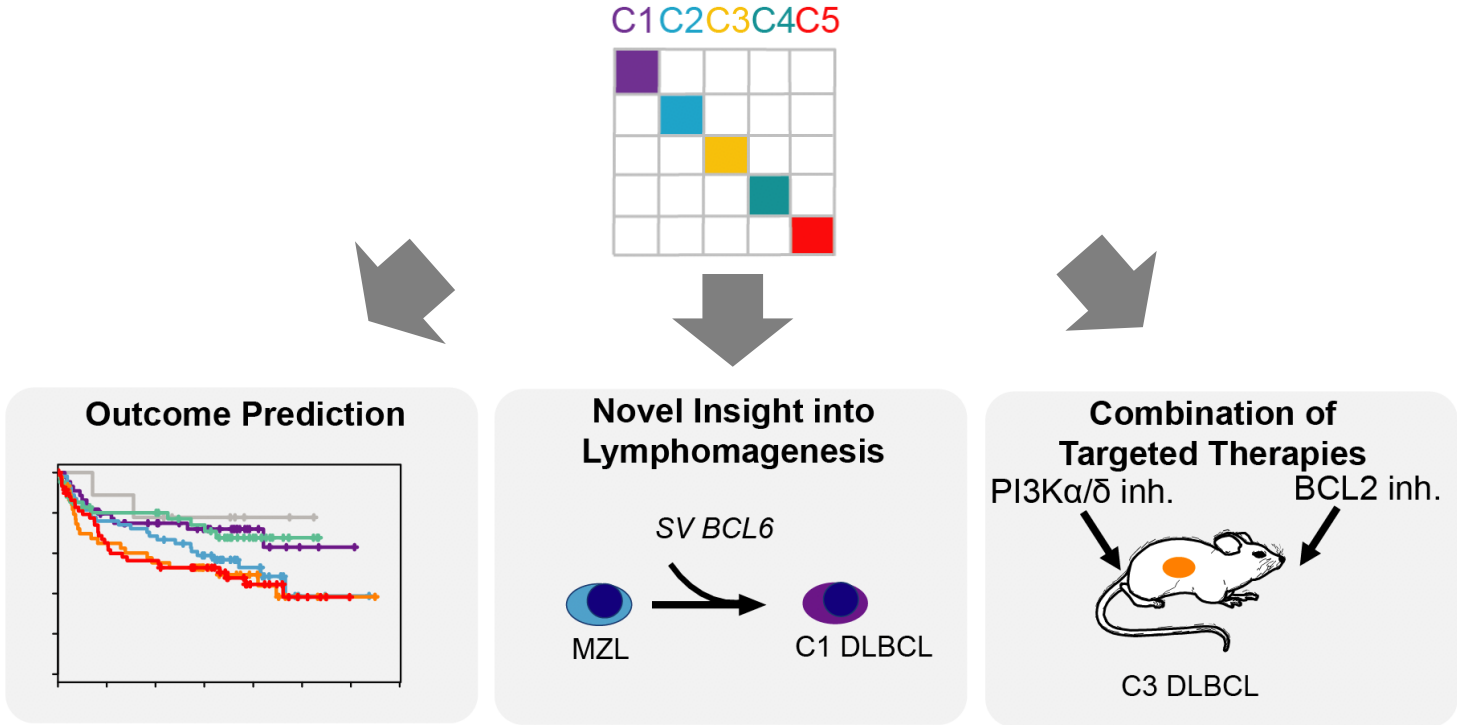
➔ Accurate identification of the C1-C5 DLBCL subtypes in newly diagnosed patients possible.

➔ Necessity for clinical translation.

Chapuy, et al. ASH 2020, unpublished

Genetically Distinct DLBCL Subtypes

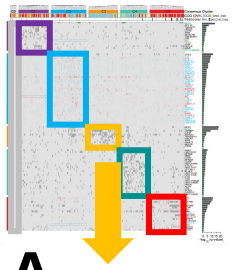




➔ Genetically-defined DLBCL subsets (C1-C5) predict different outcomes, provide novel insights into lymphomagenesis and suggest certain combinations of targeted therapies.

Chapuy, Stewart, Dunford, et al. Nat Med 2018 *Bojarczuk et al. Blood 2019* *Bojarczuk and Chapuy, EHA Educational 2019*

Roadmap to Targeted Combination Therapies – PI3Kαδ/BCL2 Inhibition in C3 DLBCLs



A

C3 DLBCLs

- Co-targeting of PI3Kad and BCL2 is highly synergistic in genetically-defined pre-clinical DLBCL models.

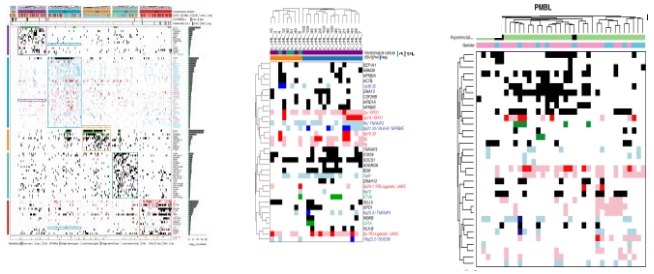
➔ Proof of concept that genetically-defined clusters provide a roadmap for rational (pre)clinical therapies

Bojarczuk K et al., Blood 2018

Molecular Lymphoma Board

~800 primary lymphoma

DLBCL **CHL** **PMBL**



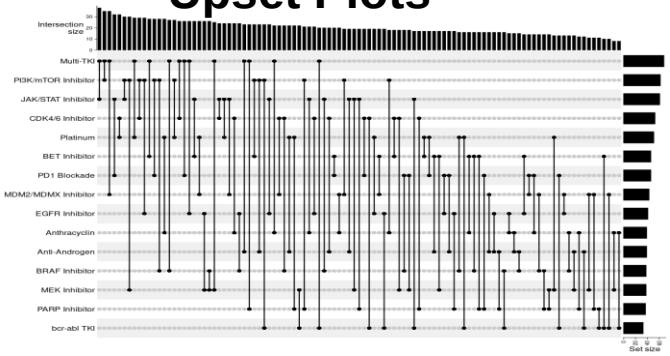
Genomic Signature

Public DBs

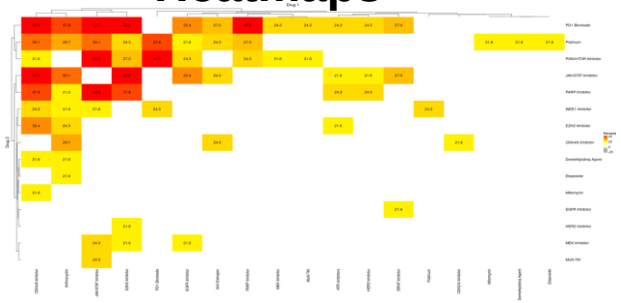
Molekular Tumorboard Onkopus

Prediction of single- and combination therapies

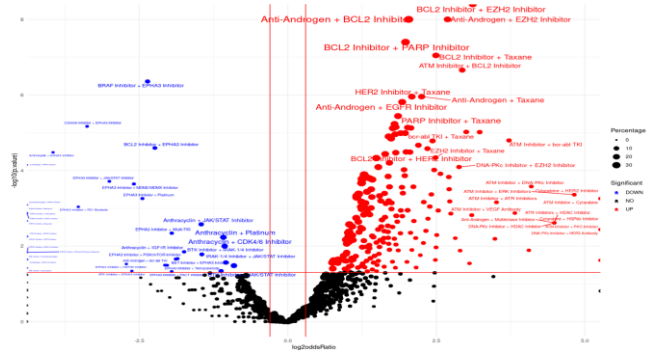
Upset Plots



Heatmaps



Vulcano Plots



SNV
CNV
Fusions

GDKD
CIViC
TARGET

Classification of therapy and evidence level

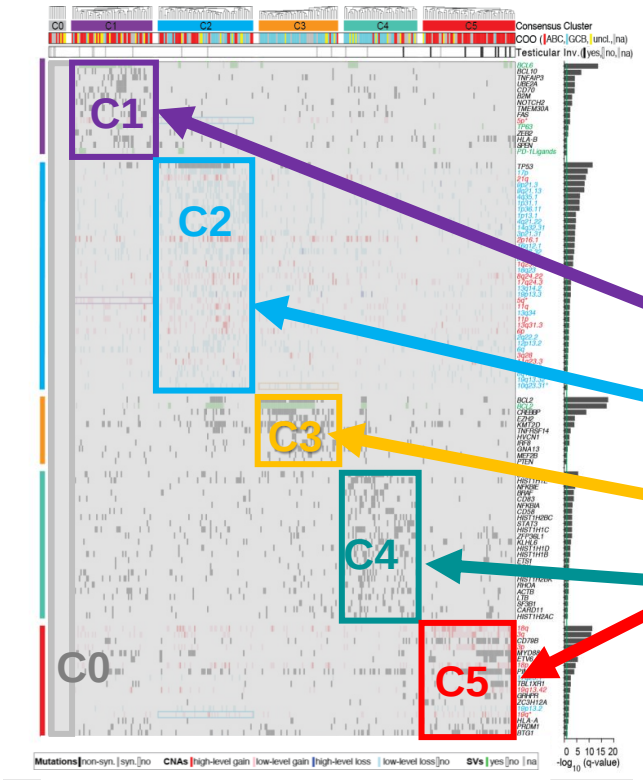
	Approved	Clinical	Preclinical
Same Cancer	A1	A2	A3
Other Cancer	B1	B2	B3

➔ Testable hypotheses are currently being evaluated in the wet-lab

In collaboration mit T. Beißbarth (UMG) und dem CADS Program des BIH

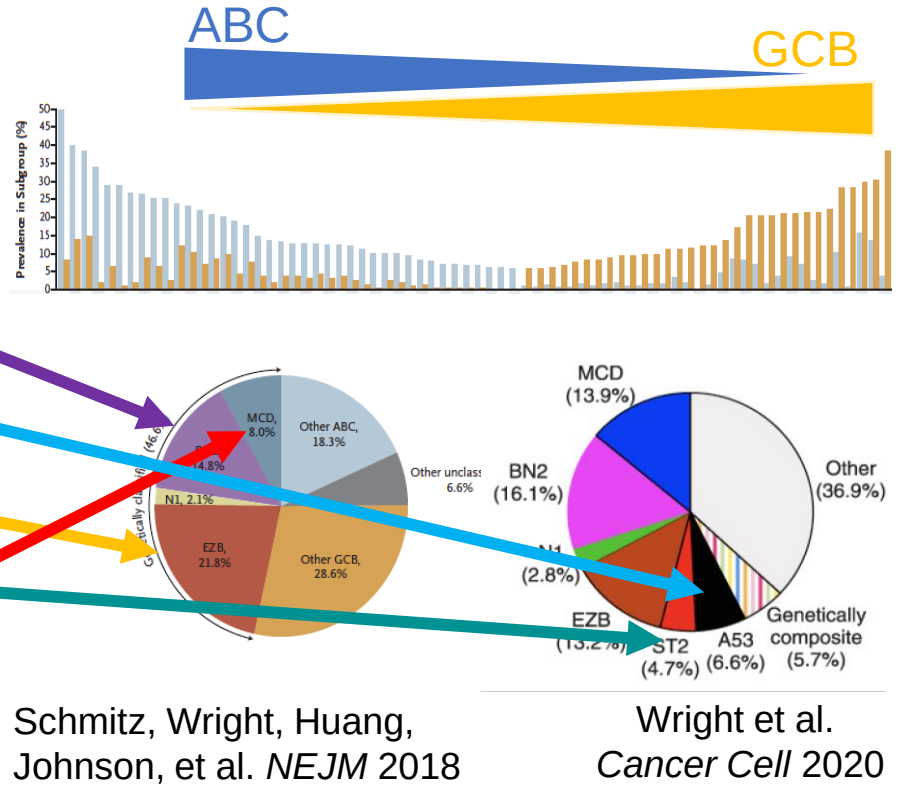
Genetic DLBCL Classifications

DLB*class*



Chapuy, Stewart, Dunford, et al. *Nat. Med.* 2018

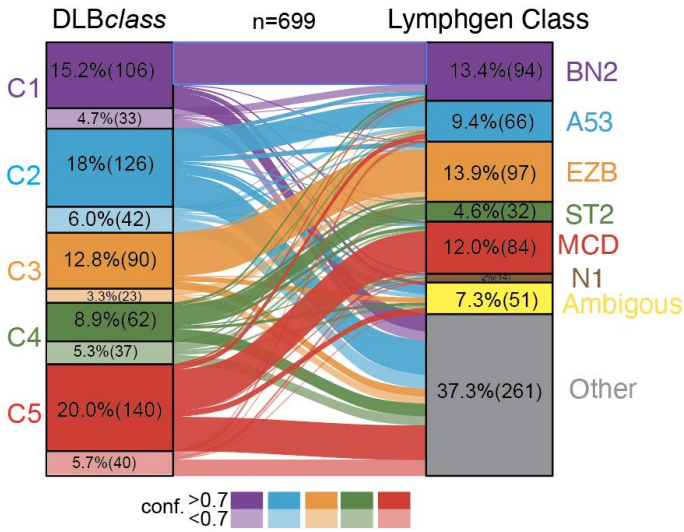
LymphGen



Schmitz, Wright, Huang, Johnson, et al. *NEJM* 2018

Wright et al. *Cancer Cell* 2020

Comparisons



unpublished

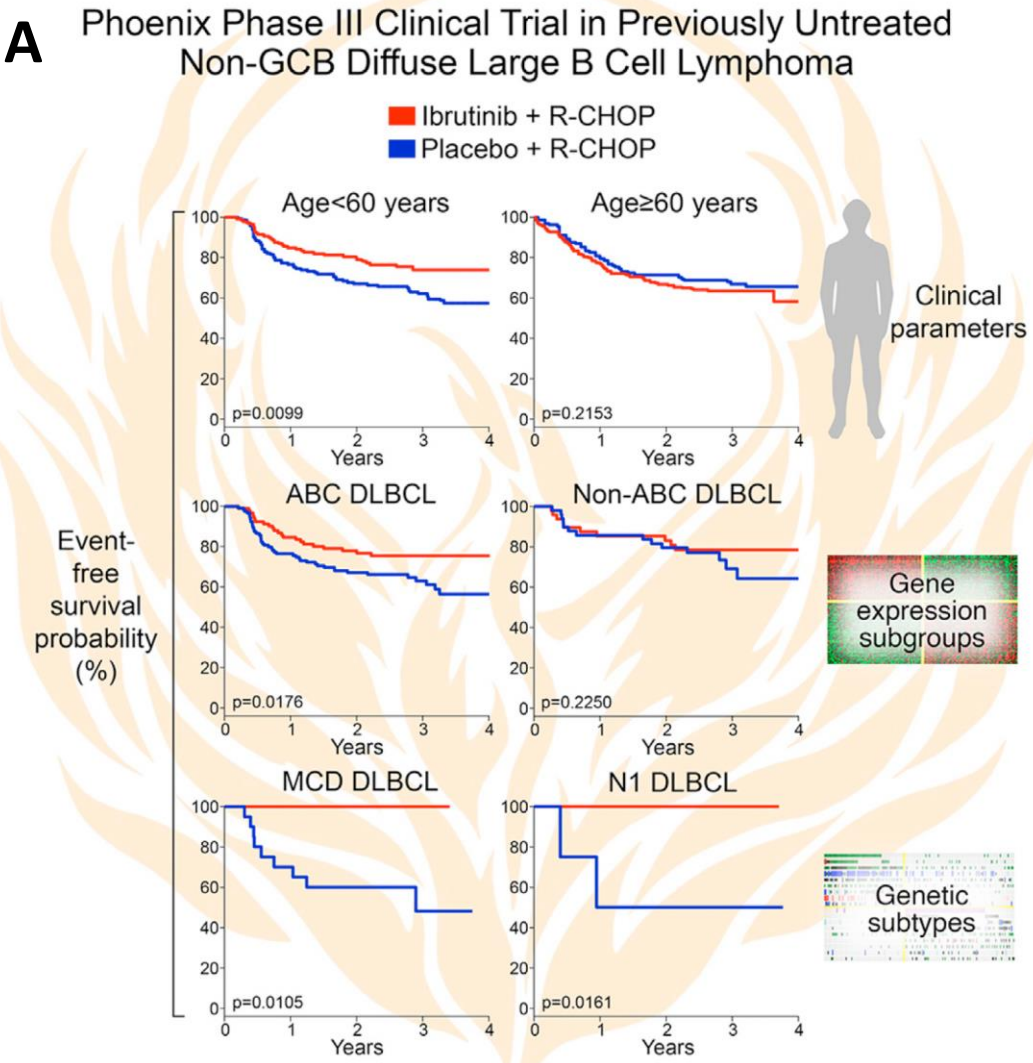
➔ DLBCL is genetically a heterogeneous disease with multiple genetic subtypes.

➔ Major subtypes have been validated using targeted approaches[1].

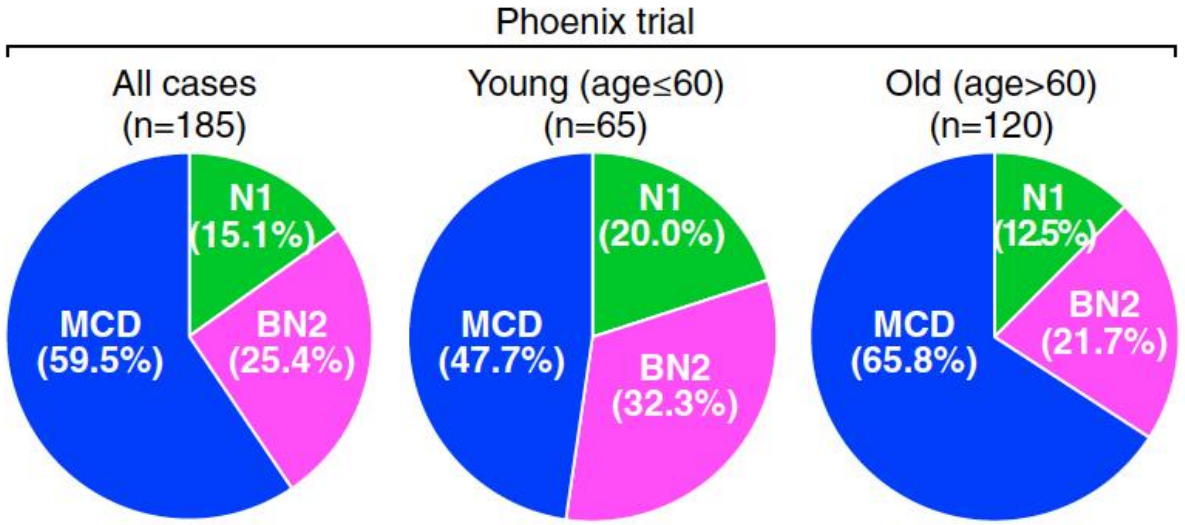
[1] Lacy et al *Blood* 2020

Effect of Ibrutinib with R-CHOP Chemotherapy in Genetic Subtypes of DLBCL

A



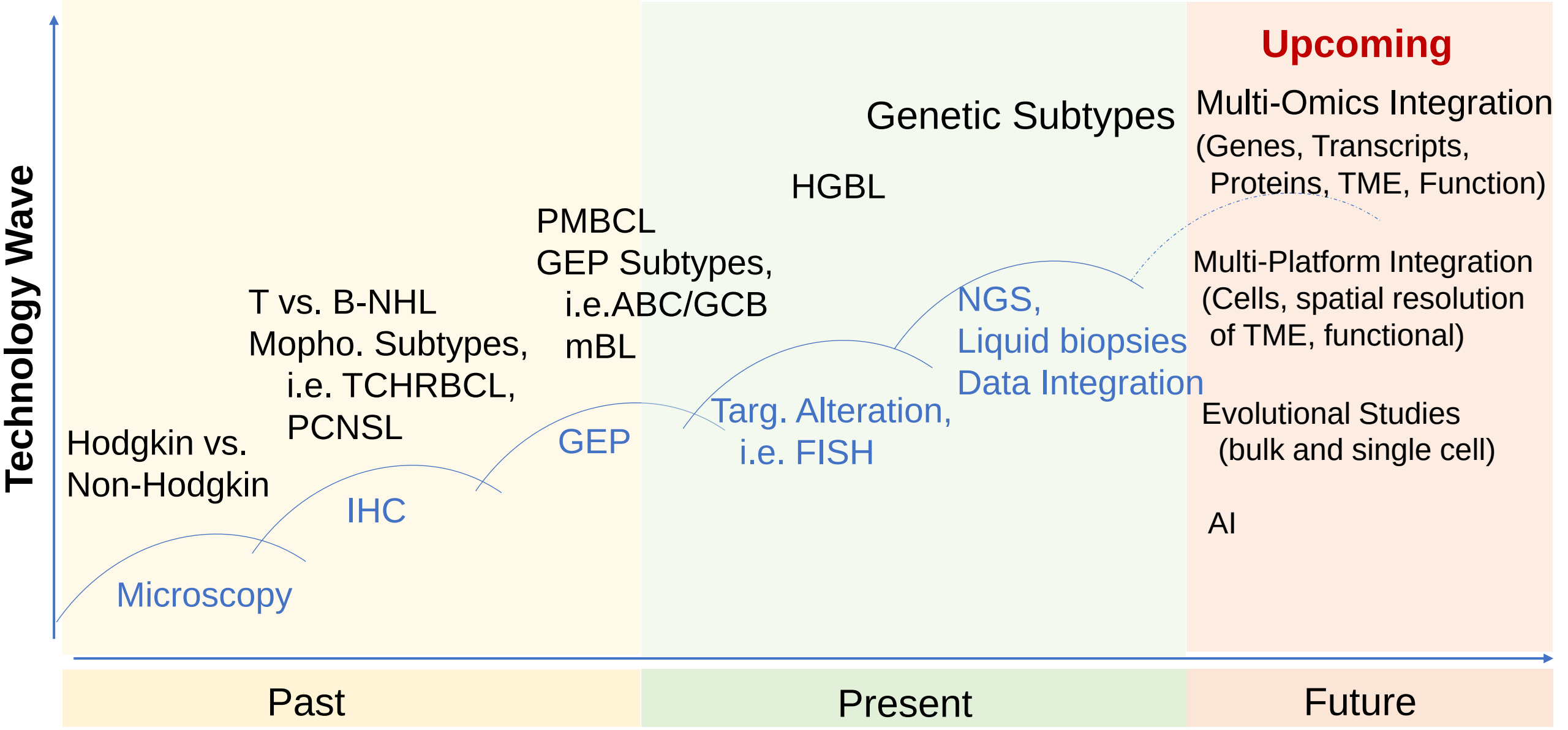
B



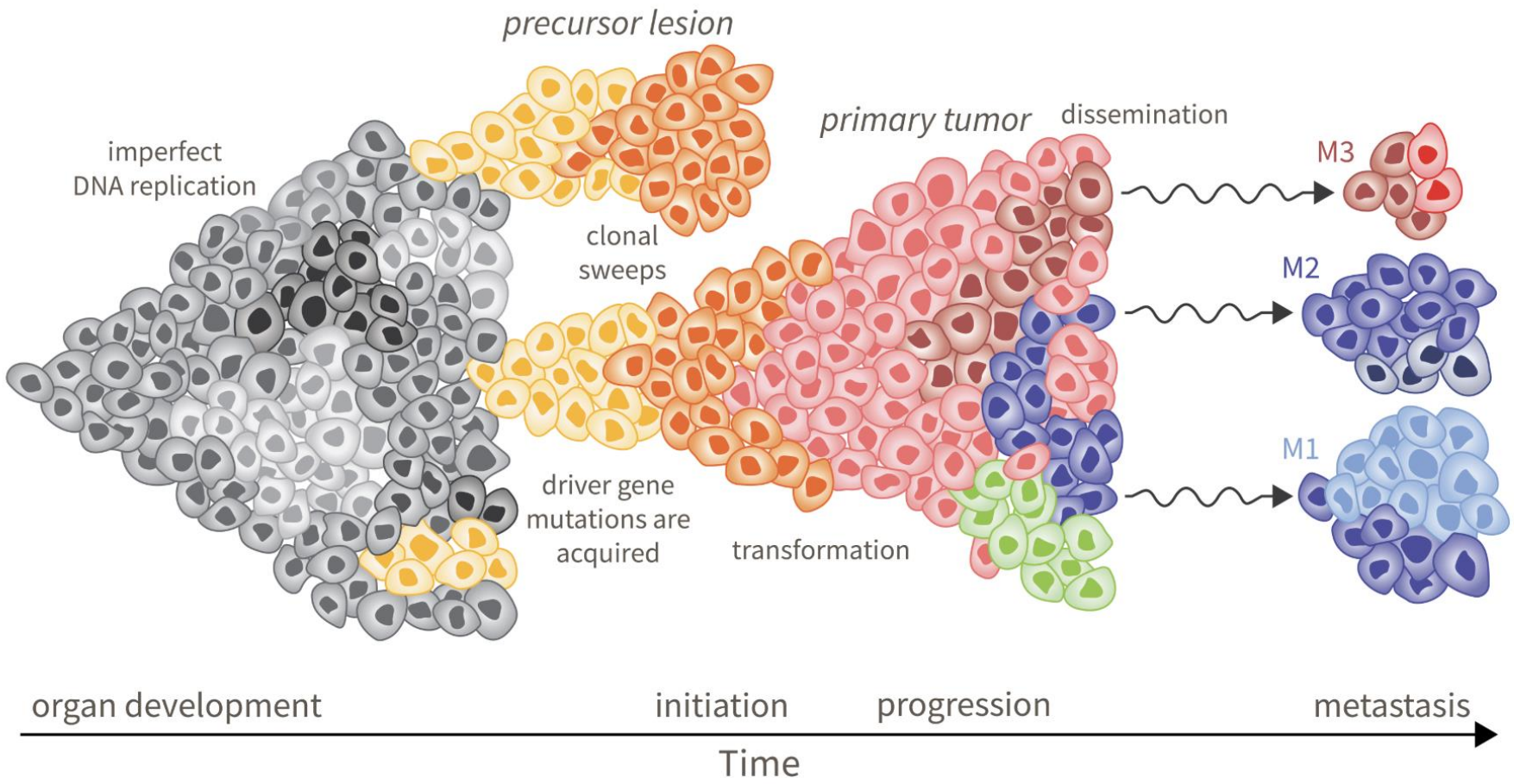
- BTK inhibitor ibrutinib plus R-CHOP is effective in younger patients with ABC DLBCL
- Patients with the MCD and N1 subtypes have 100% survival with ibrutinib plus R-CHOP

Wilson et al *Cancer Cell* 2021

Evolving Molecular Classification with Technology



Intratumoral Heterogeneioty and Clonale Evolution



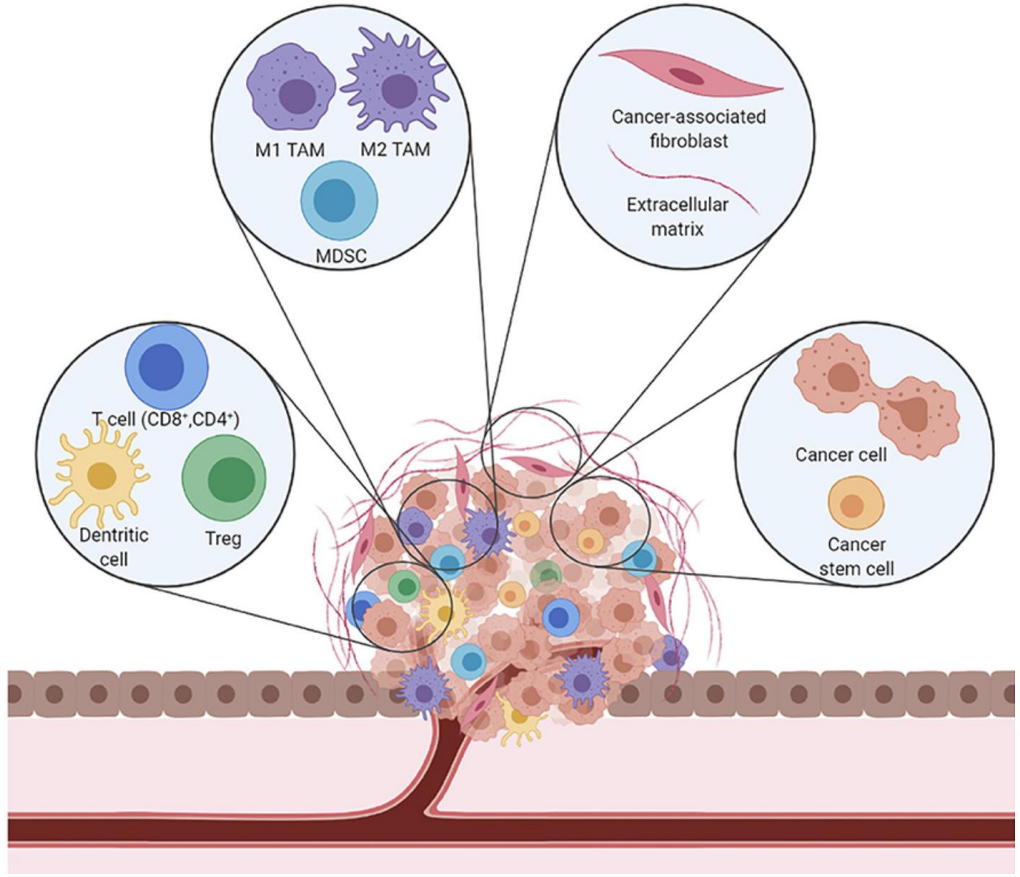
- DLBCL exhibit also intratumoral heterogeneity; Median of 17 genetic alterations.[1]
- Treatment provides selection pressure.
- Systematic analyses are clinically warranted

[1] *Chapuy, Stewart, Dunford, et al. Nat Med 2018*

Reiter et al NRC 2019 | https://reiterlab.stanford.edu/

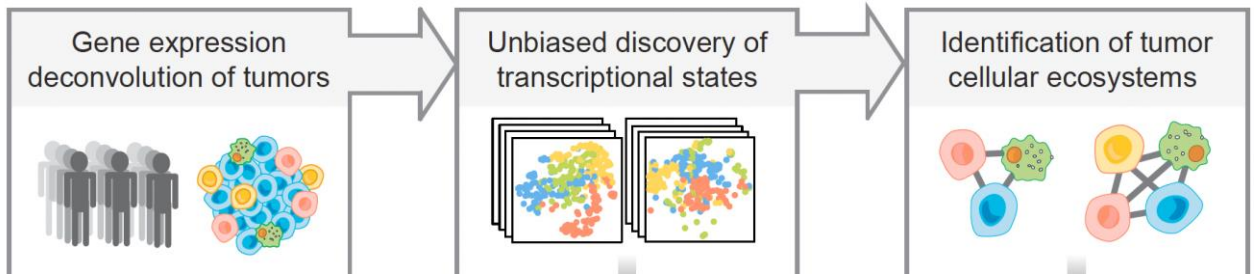
Beyond the Lymphoma Cell - Tumor as Organs

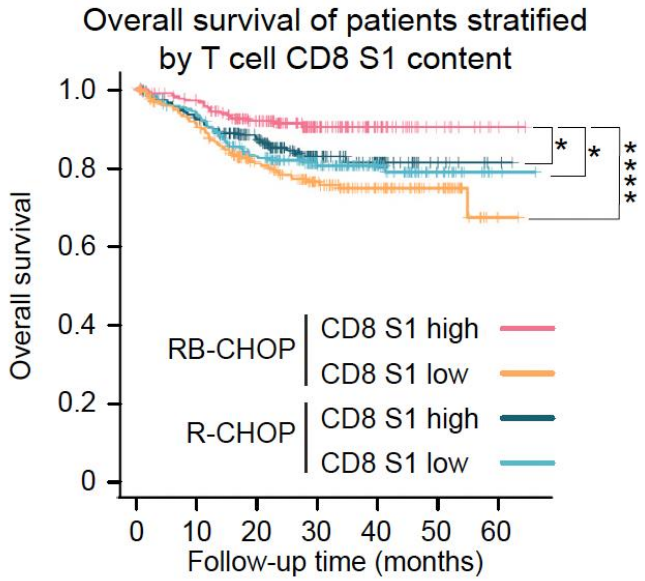
Lymphoma Microenvironment



Benavente et al *Front in Oncol* 2020

"DLBCL Ecosystems"





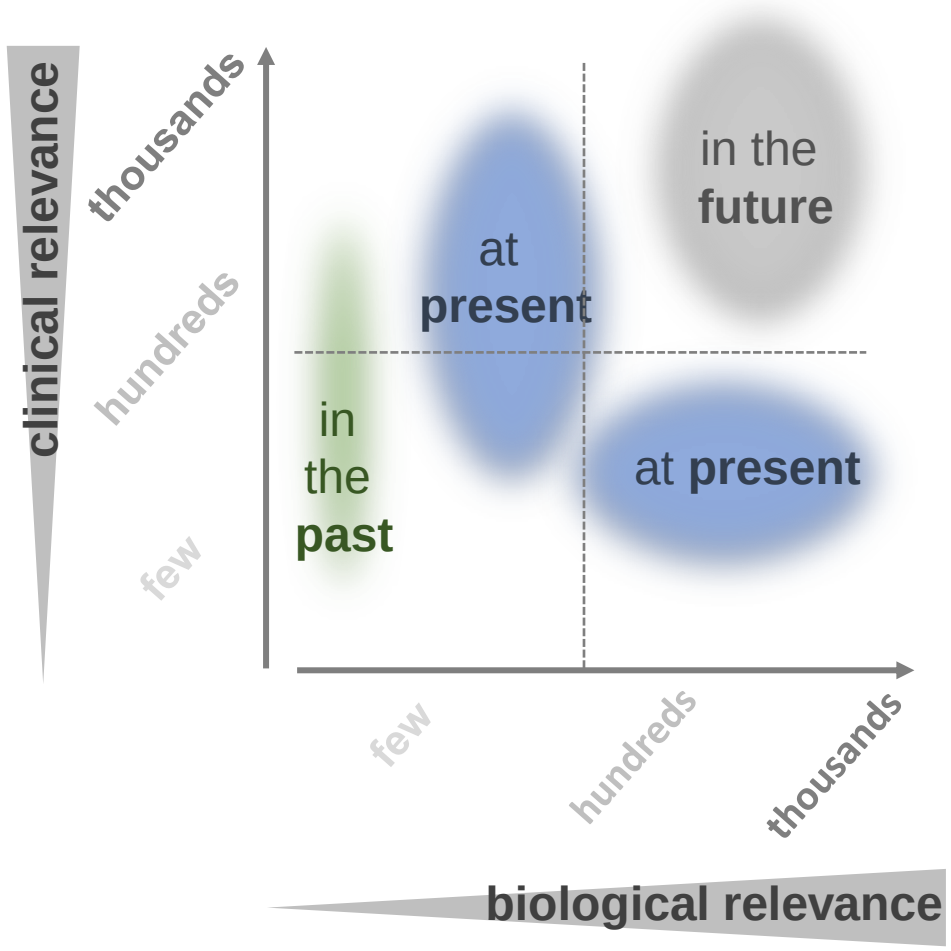
Steen, Luca et al *Cancer Cell* 2021

➔ Different lymphoma microenvironment signatures exists that might be relevant for treatment?

Dufva, Pölönen et al *Cancer Cell* 2020; Kotlov et al Cancer Discovery 2021; Steen, Luca et al *Cancer Cell* 2021; Ye et al *Cell Rep* 2022

Current Challenges with Molecular Classifiers

Evolving Approaches/"Moving Target"



Current Challenges

(1) Moving Target

(2) Limited resources

Biomaterial, people, funding

(2) Demanding/integrative computations

Expertise in bioinformatics, statistics

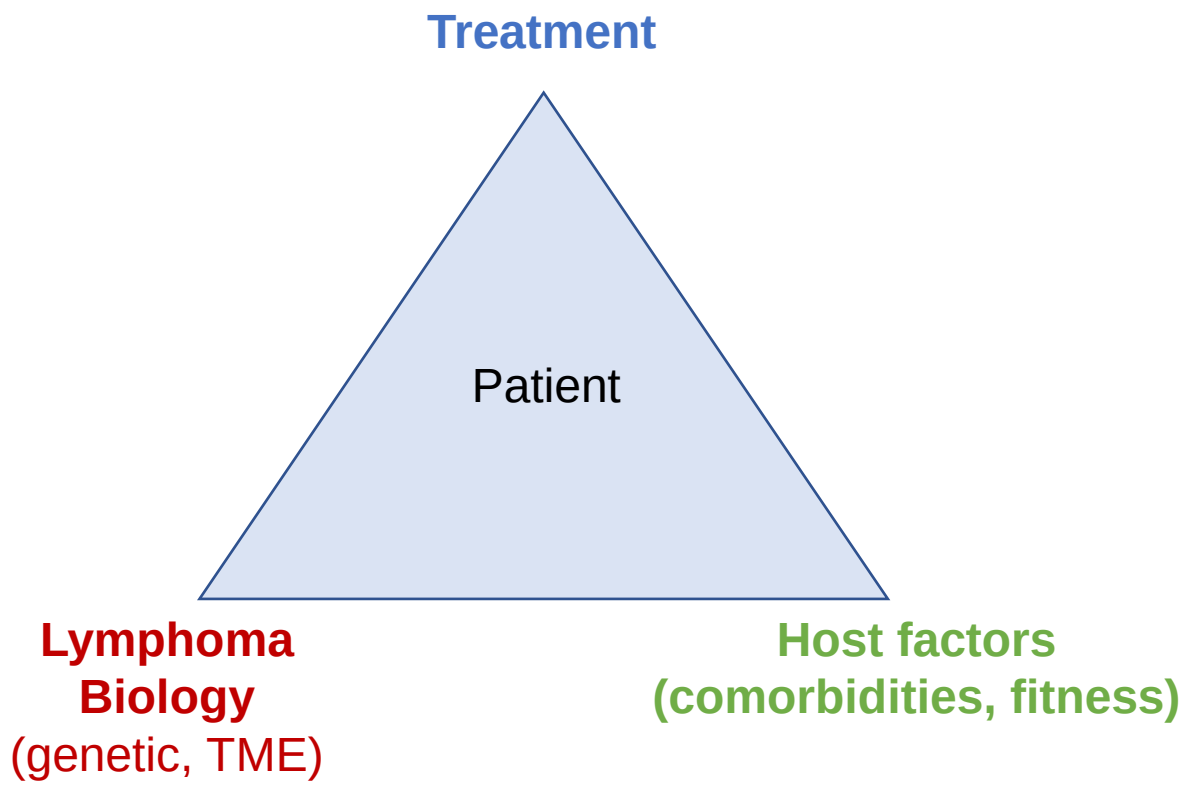
(3) Translational/clinical impact

Functional characterization

Translation into clinical trials

Weigert and Chapuy, *Onkologe 2019*

Understanding Response



Strategies Towards Precision Medicine (in Lymphoma)

Molecular agnostic

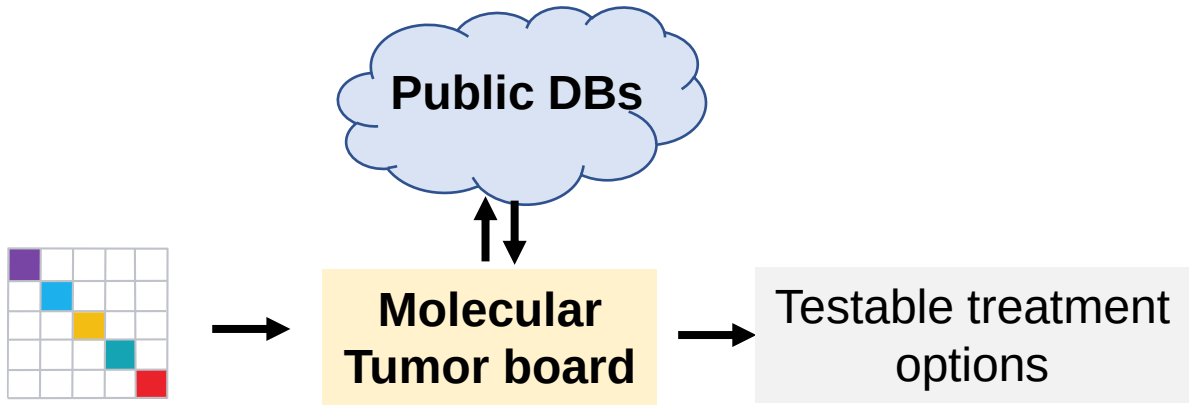
“All comer” Studies
oriented towards surface epitopes

Molecular affine

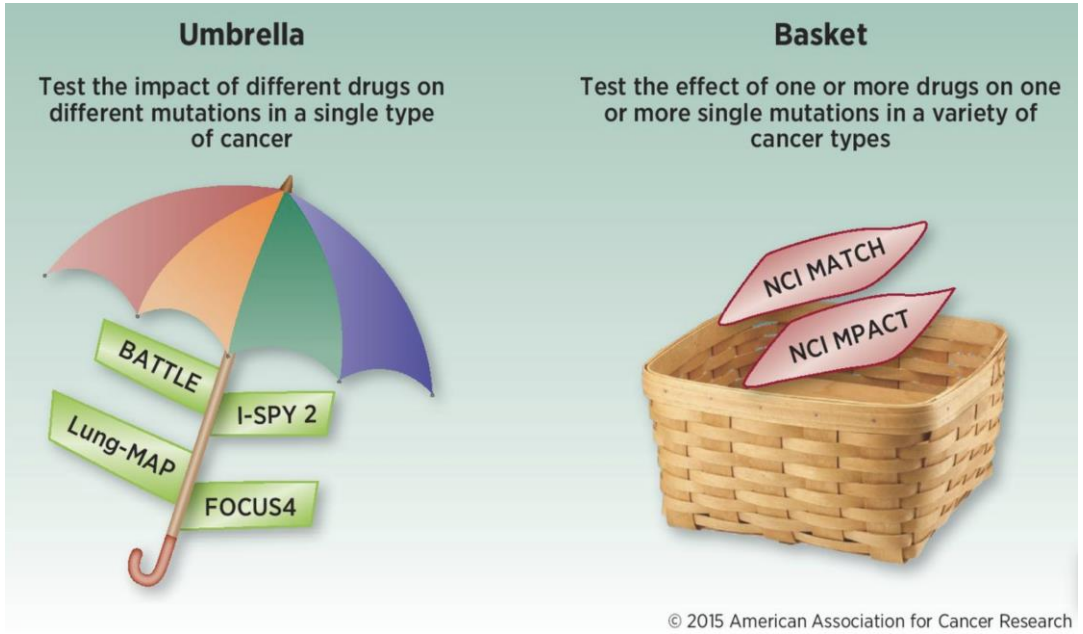
Understanding Molecular Heterogeneity
&
Targeting Actionable Alterations

Change in Patient Management and Trial Culture

Molecular Tumor Boards



All Comer Trials become problematic



➔ Complex biology demands molecularly trained physician and clinically trained biologists/computational biologists

➔ Need to rethink clinical trial designs

Biomarker-guided Targeted Therapy in DLBCL – R-CHOP+X

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.

Untreated DLBCL

- Age 18-80
- IPI ≥ 2

→ R-CHOP×1 → R 1:1

Stratified by K-medoids algorithm (PAM) simulated genetic subtyping using targeted sequencing panel of 18 genes: *BTG1, CD70, CD79B, CREBBP, DTX1, EP300, EZH2, MPEG1, MTOR, MYD88, NOTCH1, NOTCH2, PIM1, STAT6, TBL1XR1, TNFAIP3, TNFRSF14,* and *TP53*

- MCD like: Ibrutinib+R-CHOP×5
- BN2 like: Ibrutinib+R-CHOP ×5
- N1 like: Lenalidomide+R-CHOP×5
- EZB like: Tucidinostat+R-CHOP×5
- TP53 mutated: Decitabine+R-CHOP×5
- Others: Lenalidomide+R-CHOP×5

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

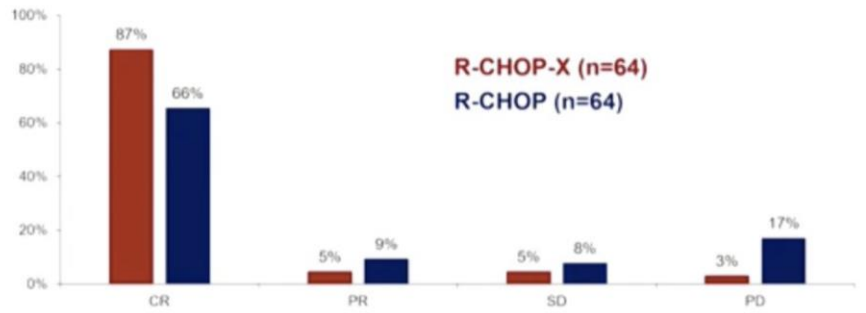
G-CSF prophylaxis was given from the second cycle of chemotherapy if grade ≥ 3 neutropenia was present in the first cycle.

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

Biomarker-guided Targeted Therapy in DLBCL – R-CHOP+X

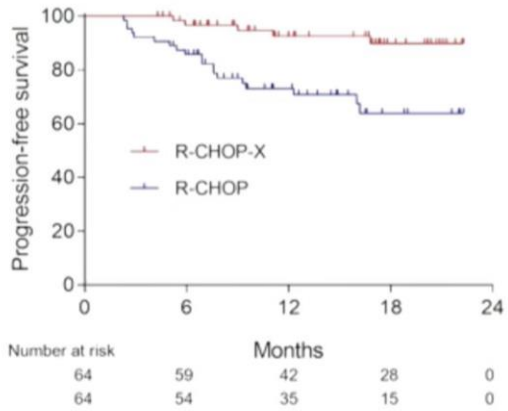
Primary Endpoint: CR rate



	R-CHOP-X	R-CHOP	P value
CRR, % (95%CI)	87 (79-96)	66 (54-78)	0.003

- The study met the prespecified primary endpoint.

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%)

Adverse Events

Toxicity	R-CHOP-X	R-CHOP
Treatment related grade 3-4 AEs (%): Hematological		
Neutropenia	81%	75%
Thrombocytopenia	31%	11%
Anemia	25%	20%
Febrile neutropenia	20%	11%
Treatment related grade 3 AEs (%): Non-hematological		
Lung infection	6%	4%
Gastrointestinal bleeding	2%	3%
Interstitial lung disease	3%	2%
Sepsis	2%	0

- Cytopenia and thrombocytopenia were common in R-CHOP-X arm but manageable with supportive care and without treatment related mortality.

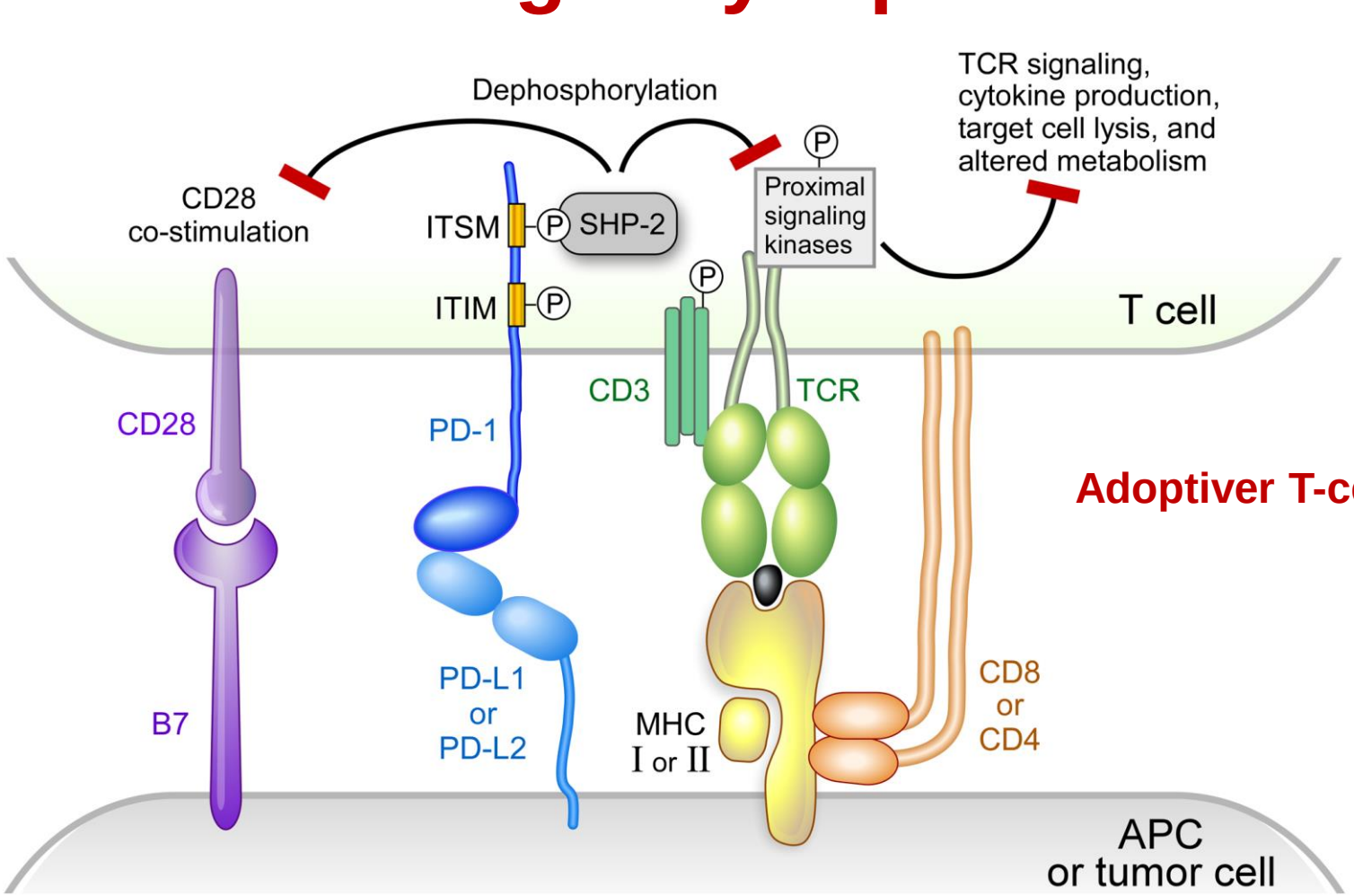
➔ Promising preliminary data

➔ Provides insights into feasibility of biomarker driven trials

➔ Follow up / full publication

Zhang et al ICML 2021

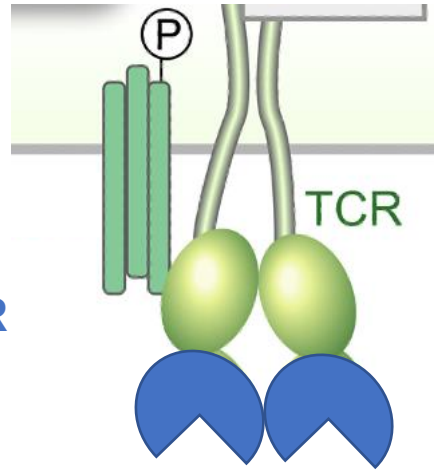
Immunologic Synapse – T-cell Activation



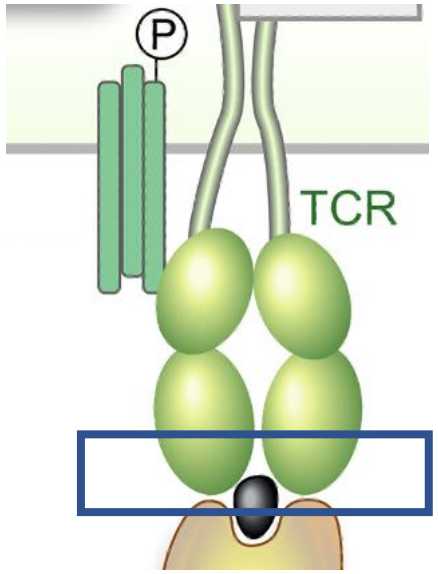
Modified from Baumeister *et al.* 2016; *Annu. Rev. Immunol.* 34:539-73

Adoptiver T-cell Transfer

CAR



TCR



Off-the-shelf TCRs in Development

Precision immunotherapy with a *MyD88 L265P* specific TCR für R/R lymphoma

Preclinical development

Clinical development

Isolation

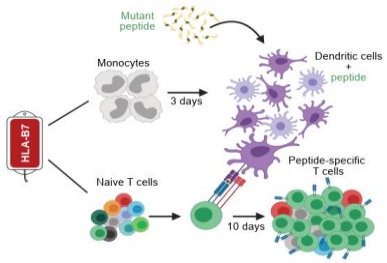
In vitro efficacy / safety testing

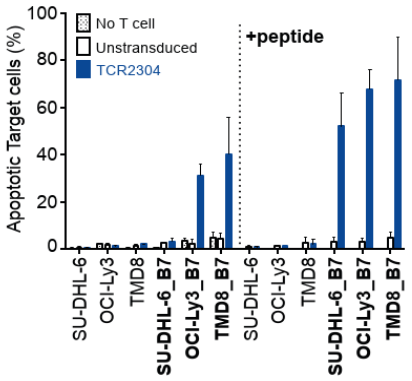
In vivo testing

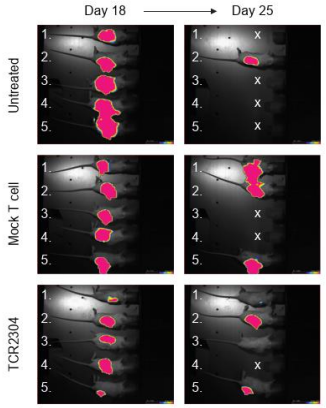
Manufacturing (Prodigy)

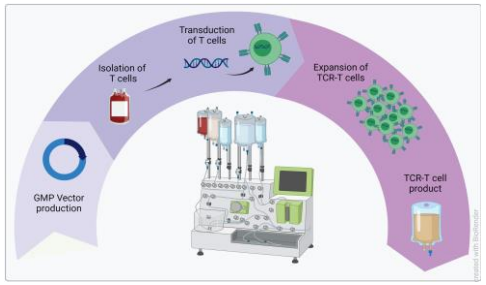
NCT Multicenter Trial

Human, HLA-matched









Auto-TCR-T-cells retroviral transduction

Charité-SCF

Berlin

Heidelberg

Würzburg

GLA

BMBF funding

First-patient-in Q1/2024

International PCT-application PCT/EP2020/051405, Cinar et al., JITC 2021

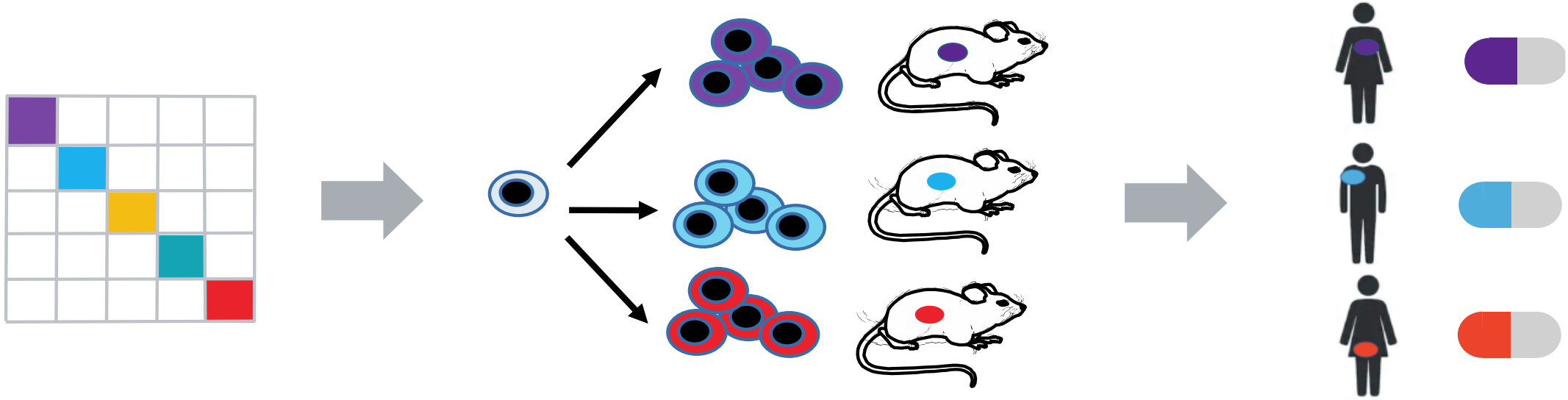
Antonia Busse

From Risk-Adapted to Biological-Informed Lymphoma Therapies

Identify Actionable Molecular Signatures

Exploit Associated Survival Pathways

Develop Rational Therapies and Biomarkers



Interested?

Contact bjoern.chapuy@charite.de

More Info https://go.umg.eu/ag-chapuy

Team Effort – The Chapuy Laboratory

Hiring now! Open Positions for:
PhDs, Postdocs and Computational Biologists

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Manfred Konrad
Joji Shimono
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Clinical Scientists
Hannes Treiber
Markus Maulhardt
Rebecca Wurm-Kuczera
David Böckle

PhD
Nazli Serin
Debora Joopi
Luisa Ohlmeier

MTAs
Jennifer Günther
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Cand Meds/Master
Jonas Brandes
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Marek Werth

More Info https://go.umg.eu/ag-chapuy

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Thank you for your attention!

Looking forward to your questions?

Contact info:
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