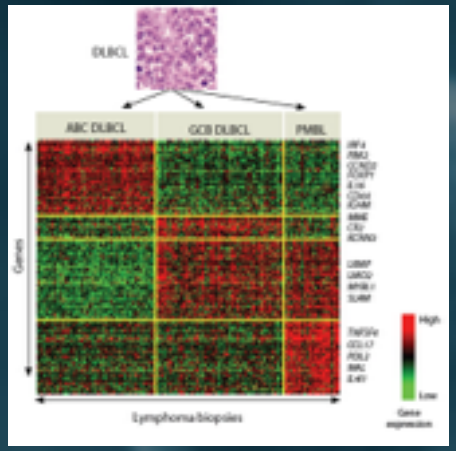
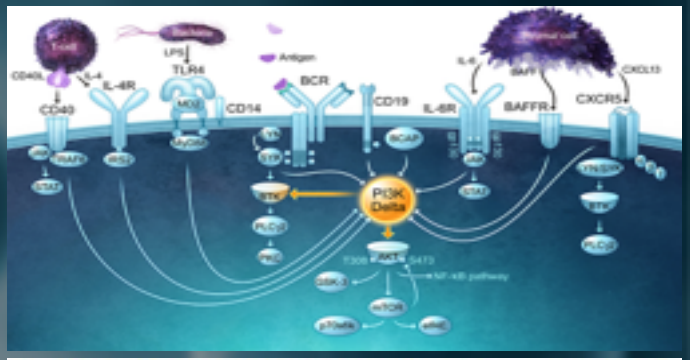
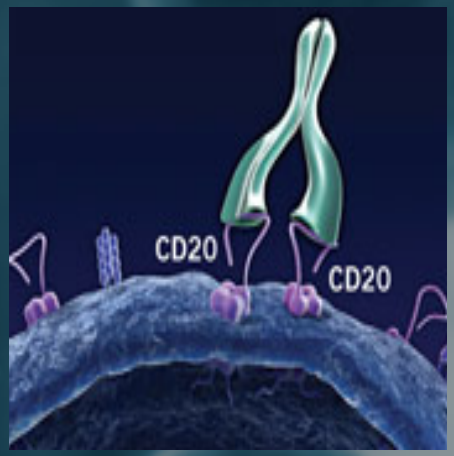
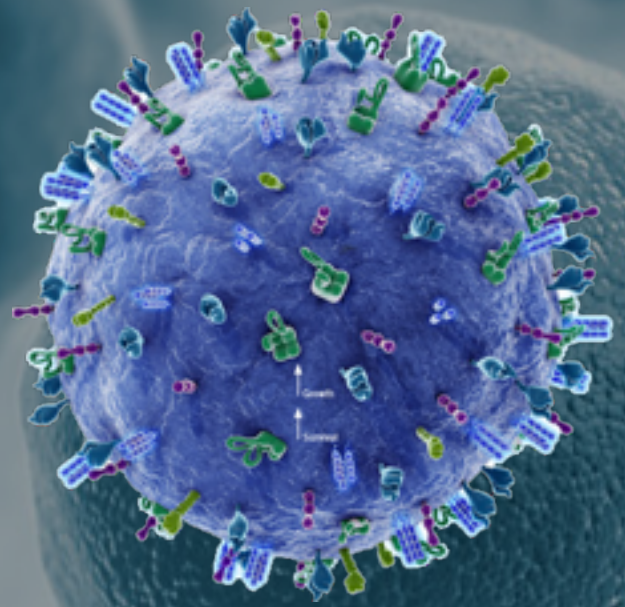
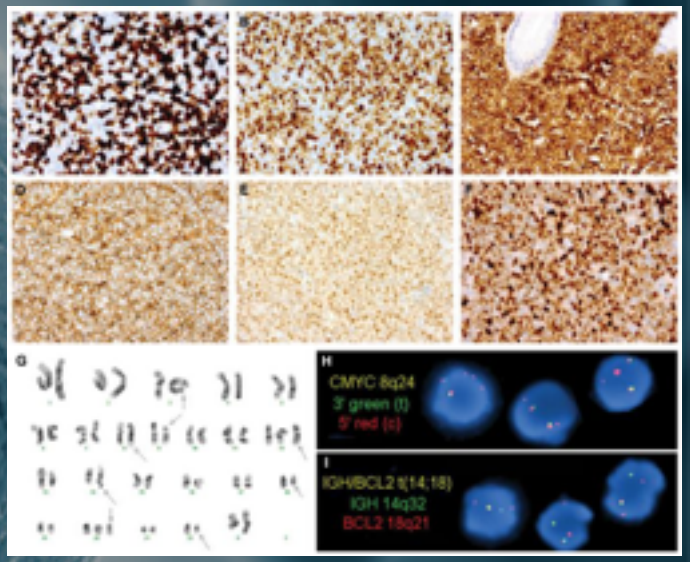










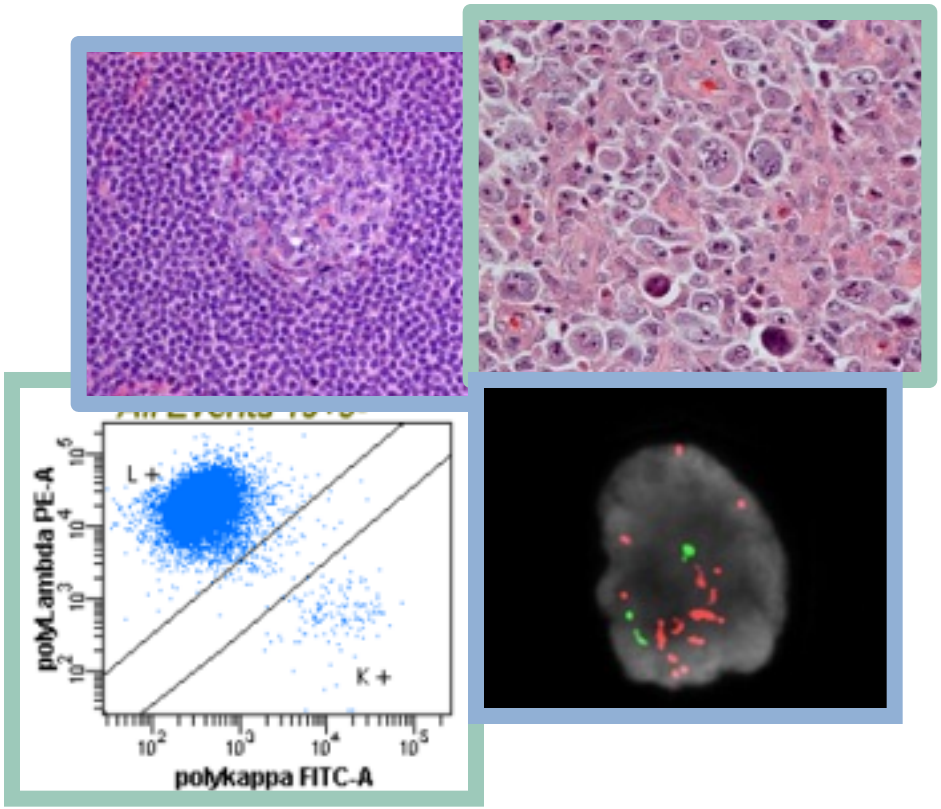
How to incorporate data from molecular studies (I.e. Gene expression profiling, FISH) into the front-line and second-line therapy for lymphoma patients

Francisco J Hernandez-Ilizaliturri MD
Chief of the Lymphoma and Myeloma Section
Professor of Medicine
Director of the Lymphoma Translational Research Program
Associate Professor of Immunology

ROSWELL PARK CANCER INSTITUTE

Can an understanding of biology improve management of lymphoma?

- Lymphoma is a heterogeneous disease with significant clinical variation
- Understanding lymphomagenesis may improve management by enabling:
  - Better classification
  - Rational treatment approaches based on disease biology



Ramsdale E et al. *Semin Oncol*. 2011;38(2):225-235.

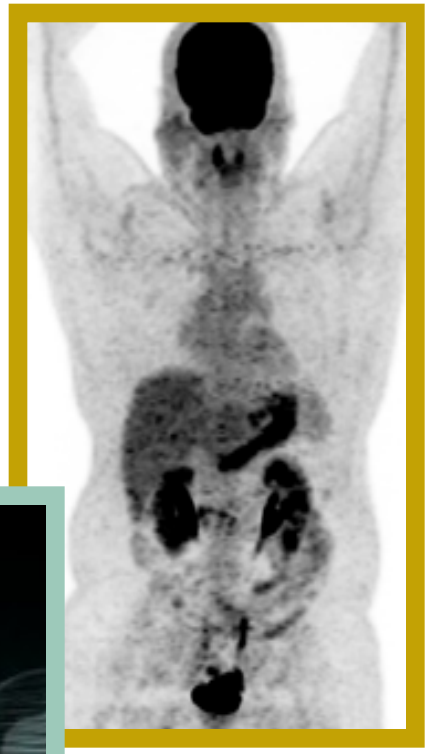
Case study: initial presentation

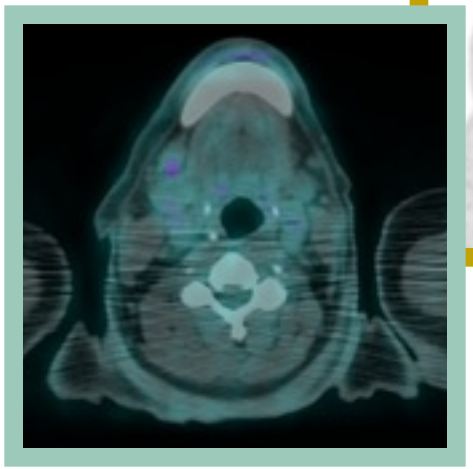
Patient

- 60-year-old African-American male

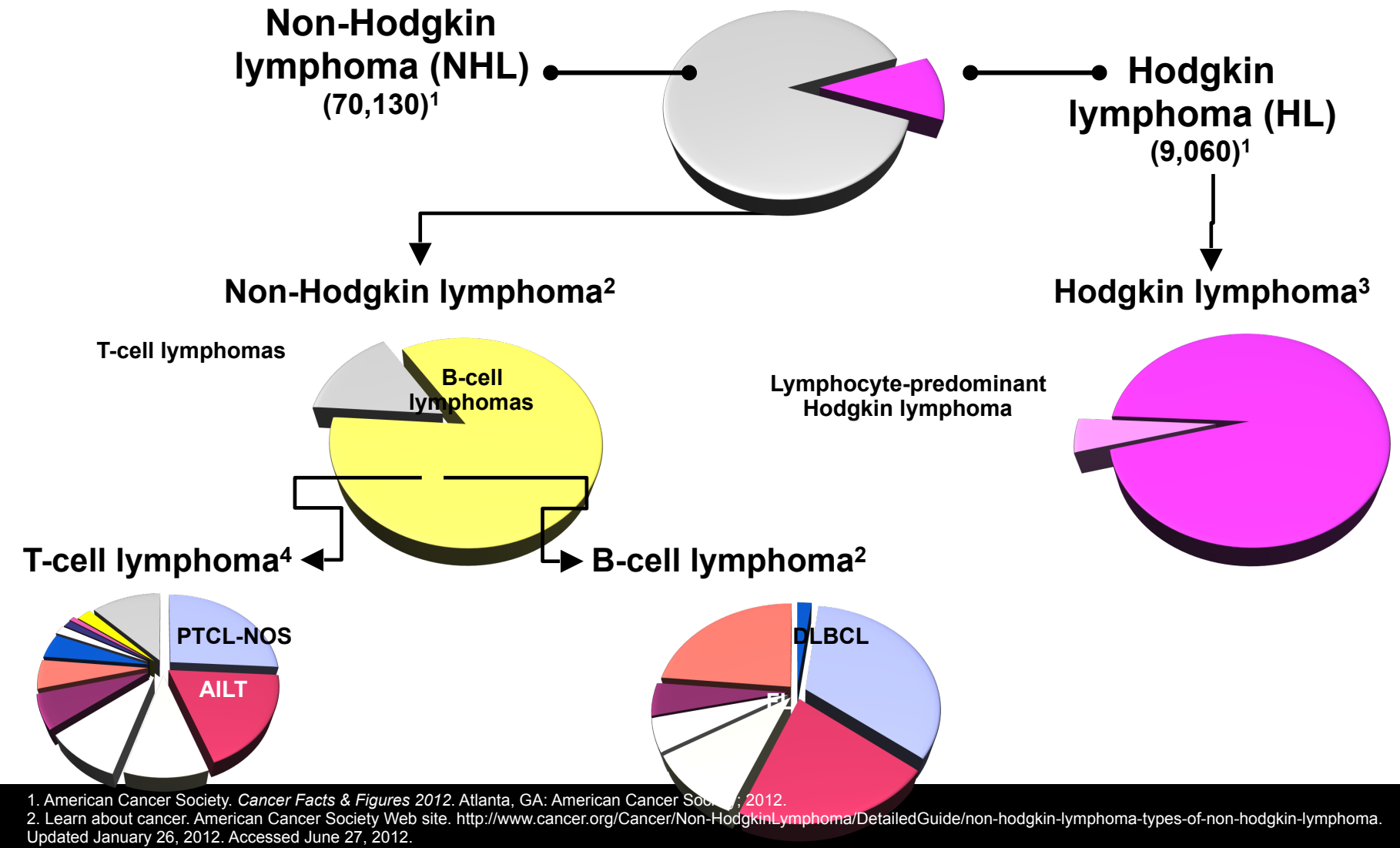
Presenting complaints

- Enlarged submandibular mass noticed 2 weeks ago during shaving
- Fatigue



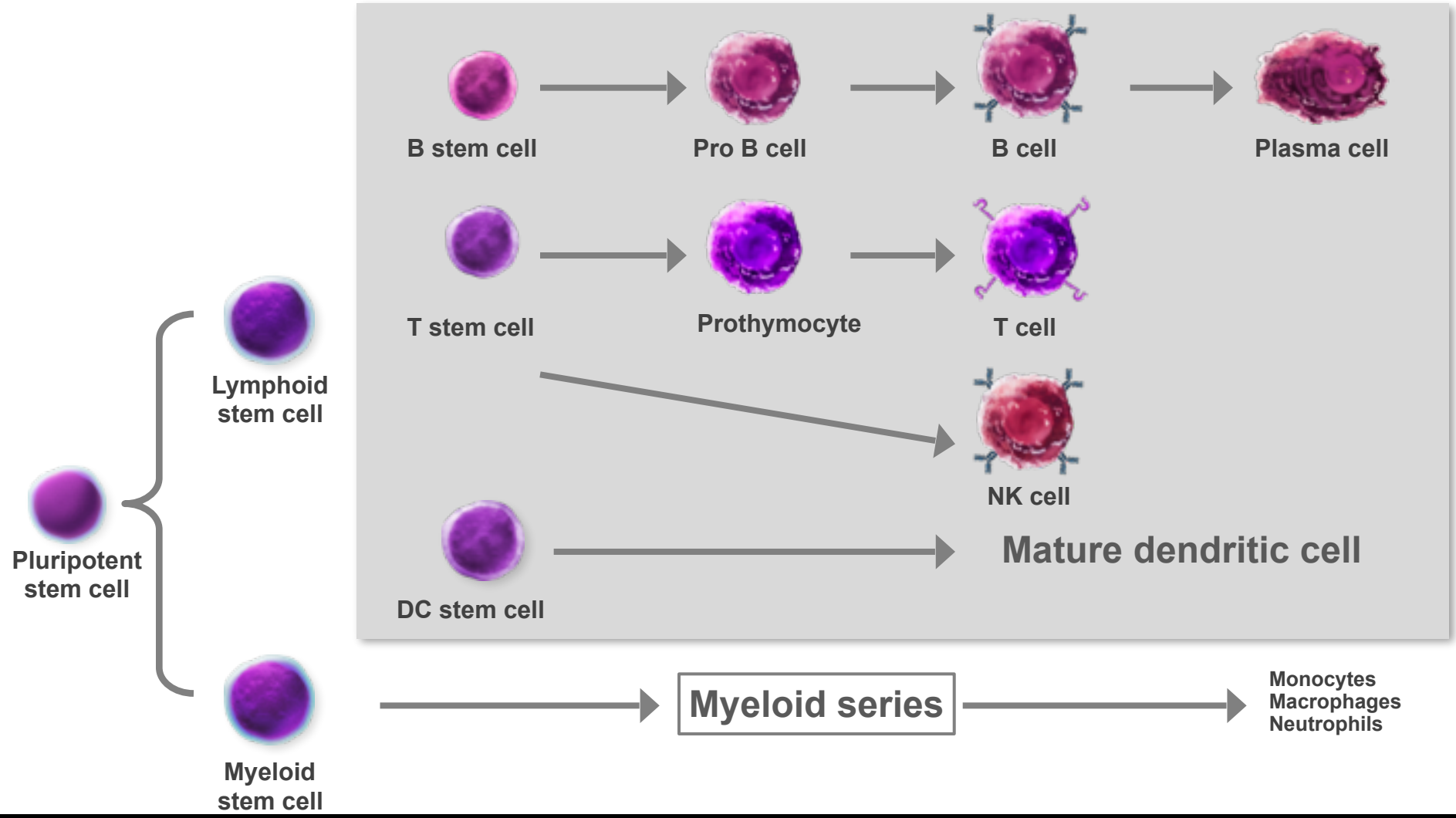


Lymphoma is a heterogeneous disease comprised of multiple subtypes



1. American Cancer Society. *Cancer Facts & Figures 2012*. Atlanta, GA: American Cancer Society; 2012.
2. Learn about cancer. American Cancer Society Web site. http://www.cancer.org/Cancer/Non-HodgkinLymphoma/DetailedGuide/non-hodgkin-lymphoma-types-of-non-hodgkin-lymphoma. Updated January 26, 2012. Accessed June 27, 2012.
3. Küppers R. *Nat Rev Cancer*. 2009;9(1):15-27.
4. Vose J, et al; International T-Cell Lymphoma Project. *J Clin Oncol*. 2008;26(25):4124-4130.

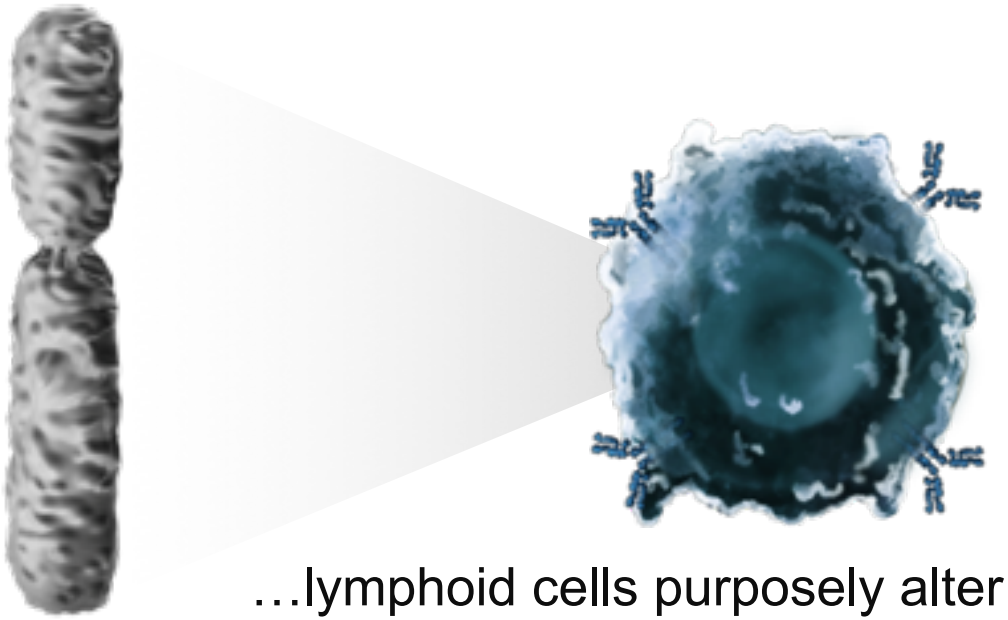
Lymphocyte development is a complex process that occurs in discrete steps[1-3]



1. Orkin SH et al. *Cell*. 2008;132(4):631-644.
2. Uckun FM. *Blood*. 1990;76(10):1908-1923.
3. Swerdlow SH et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC; 2008.

Lymphocytes Undergo Genomic Alterations During Normal Development

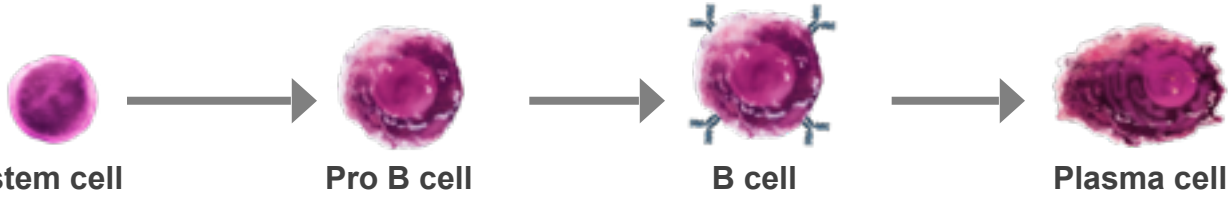
Despite the oncogenic dangers associated with genomic instability and mutation…

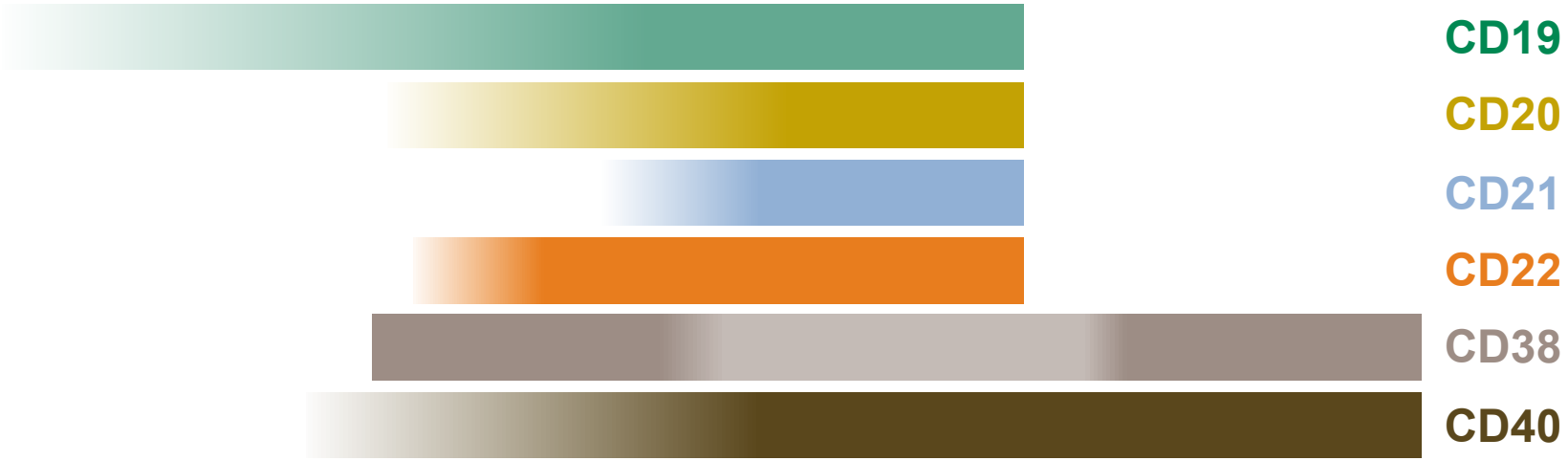


…lymphoid cells purposely alter their DNA during development to maximize the diversity and effector functions of their antigen receptors

Tsai AG , Lieber MR. *BMC Genomics*. 2010;11(suppl):S1.

Stages of B-cell development are defined by surface antigen expression[1-3]





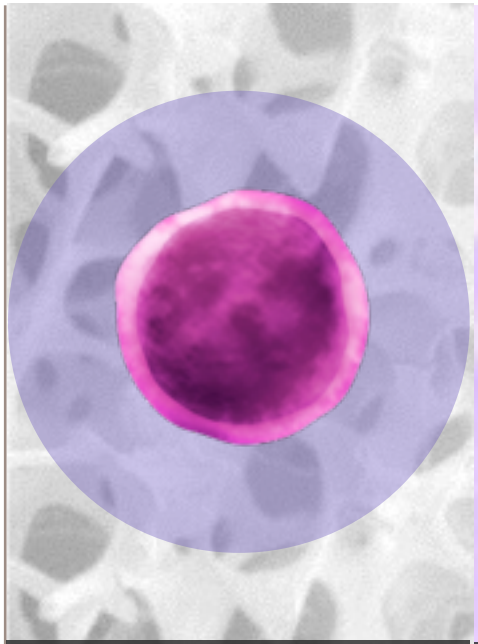
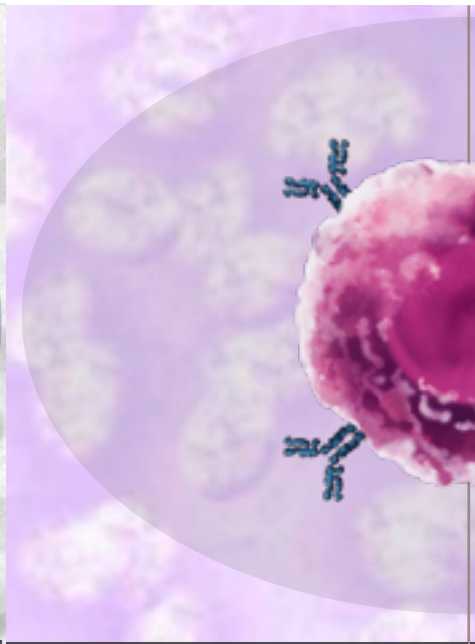
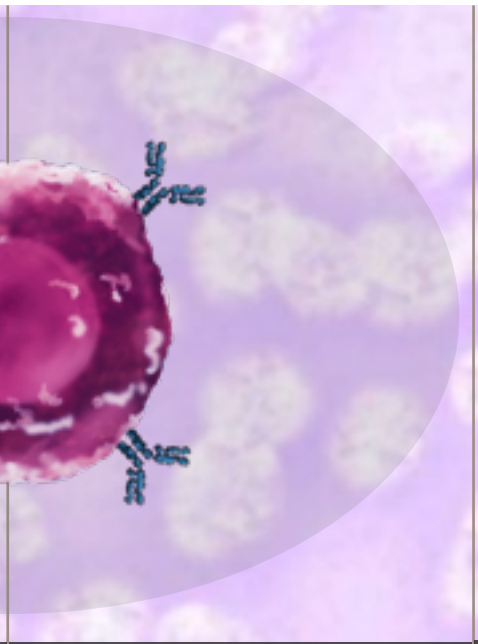
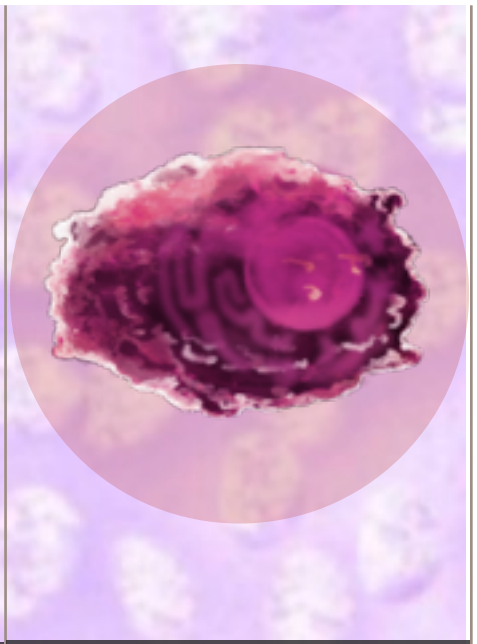
1. Orkin SH et al. *Cell*. 2008;132(4):631-644.
2. Uckun FM. *Blood*. 1990;76(10):1908-1923.
3. Swerdlow SH et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC; 2008.

Lymphoma subtypes arise from different stages of B-cell development


Pro/pre

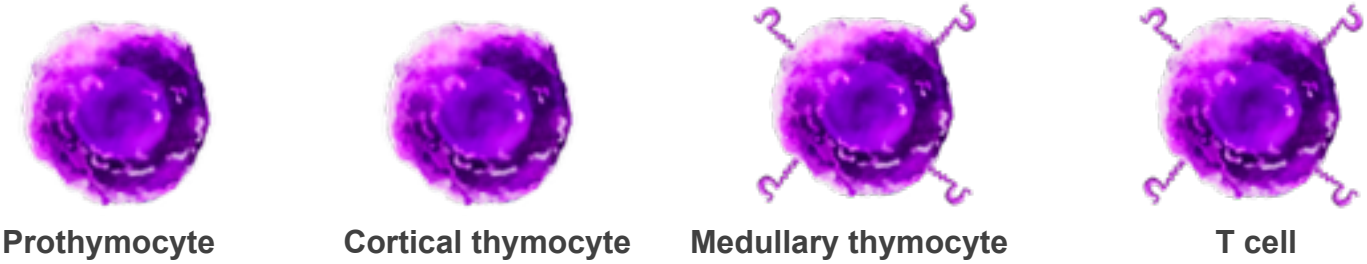

Mature


Plasma cell

Bone marrow	Interfollicular area	Follicular area	Perifollicular area
			
B lymphoblastic leukemia/lymphoma	**Mantle cell lymphoma**	**Follicular lymphoma Burkitt lymphoma DLBCL (some) Hodgkin lymphoma**	**Multiple myeloma Plasmacytoma Plasma cell leukemia**

Swerdlow SH et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC; 2008.

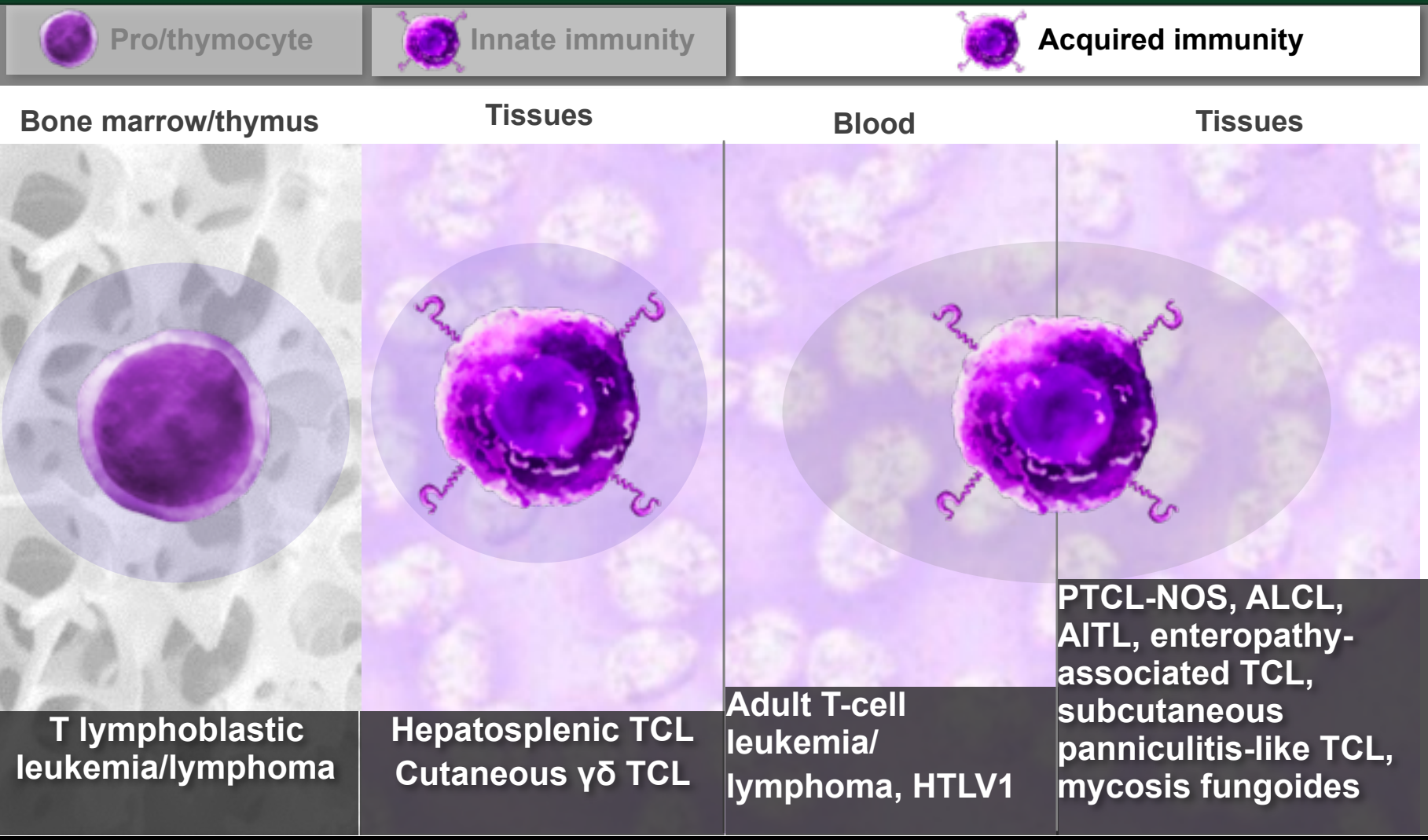
Stages of T-cell development are defined by surface antigen expression



*Terminal deoxynucleotidyl transferase.

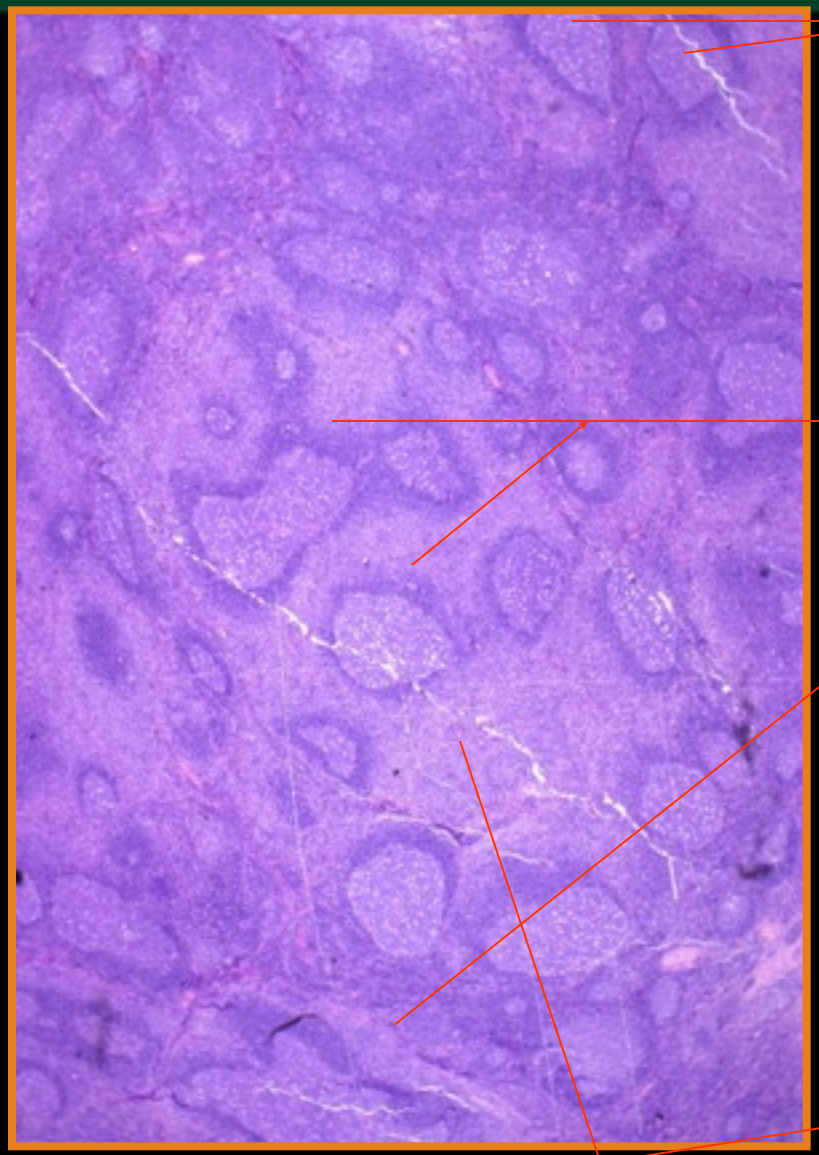
Swerdlow SH et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC; 2008.

Lymphoma subtypes arise from different stages of T-cell development[1,2]



1. Swerdlow SH et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC; 2008.
2. de Leval L et al. *Histopathology*. 2011;58(1):49-68.

Understanding lymphoma classification



Follicles made of B-cells, when malignant called B-cell follicular lymphoma

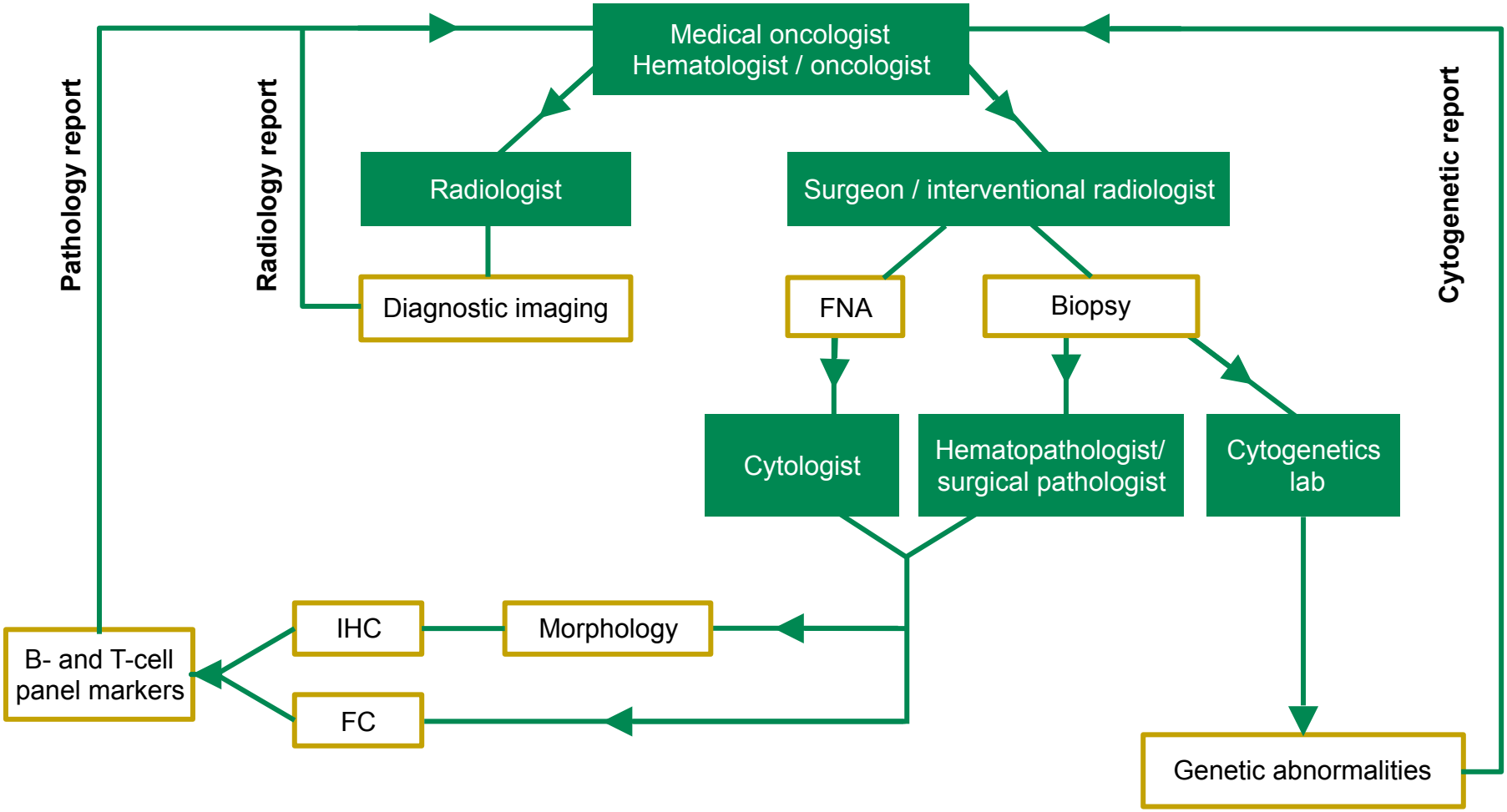
Dark rim of cells around follicles are mantle zones, when malignant, B-cell mantle cell lymphoma

Pale pink area with small cells immediately next to the manlte zone is marginal zone, when malignant marginal zone lymphoma. If this type of lymphoma is in soft tissue, extra nodal marginal zone lymphoma

Interfollicular areas composed of

T-cells, when malignant, T-cell lymphomas

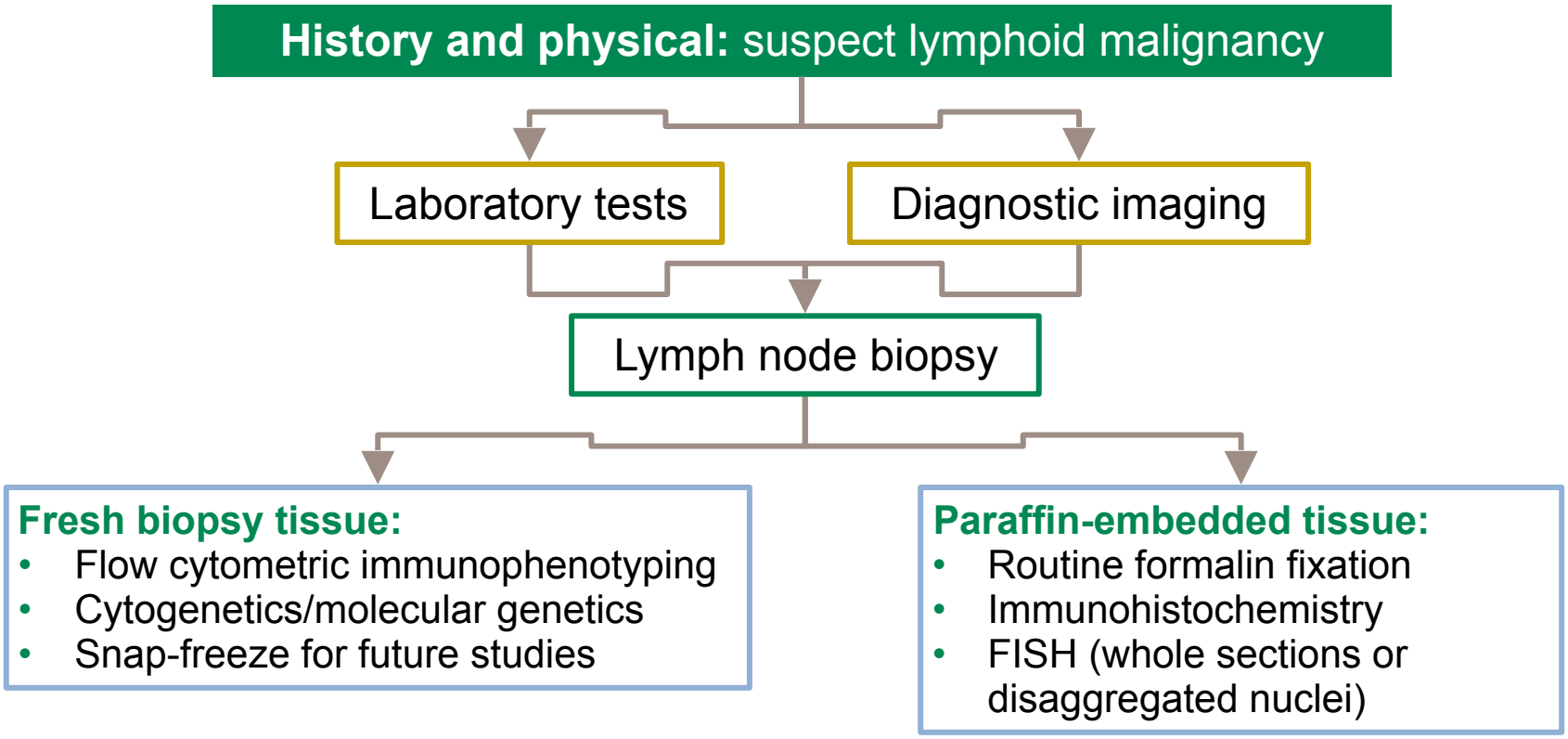
Lymphoma diagnosis requires a multidisciplinary team approach[1,2]



FC = flow cytometry; IHC = immunohistochemistry

1. Ansell SM et al. *Mayo Clin Proc.* 2005;80(8):1087-1097.
2. National Comprehensive Cancer Network (NCCN). NCCN clinical practice guidelines in oncology: non-Hodgkin's lymphomas (version 2.2012). Fort Washington, PA: NCCN; 2012.

A systematic approach to diagnosing suspected lymphoid cancers is recommended[1,2]



Is this diagnostic algorithm consistent with what is standard practice at your medical center?

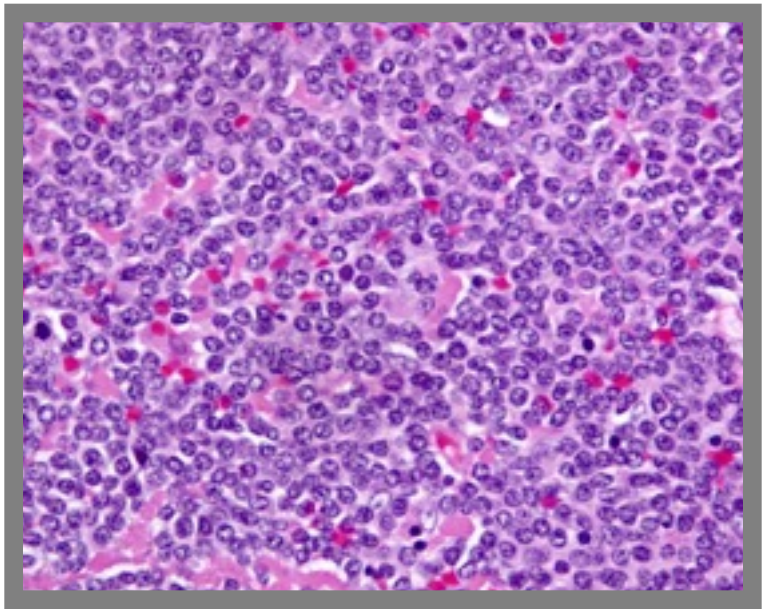
1. Armitage JO et al. *Clin Adv Hematol Oncol.* 2010;8(12)(suppl 22):1-15.
2. Wilkins BS. *J Clin Pathol.* 2011;64(6):466-476.

Are we looking at lymphoid cancer?

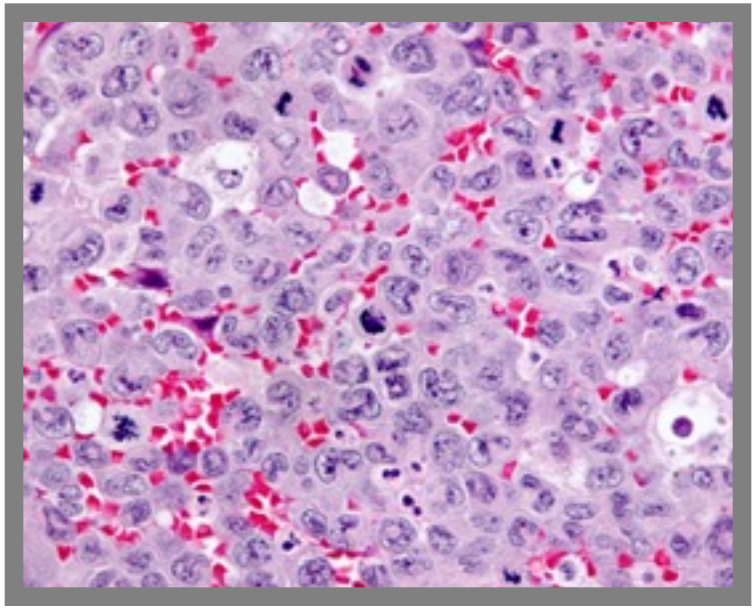
Excisional lymph node biopsy

Lymphoid cells

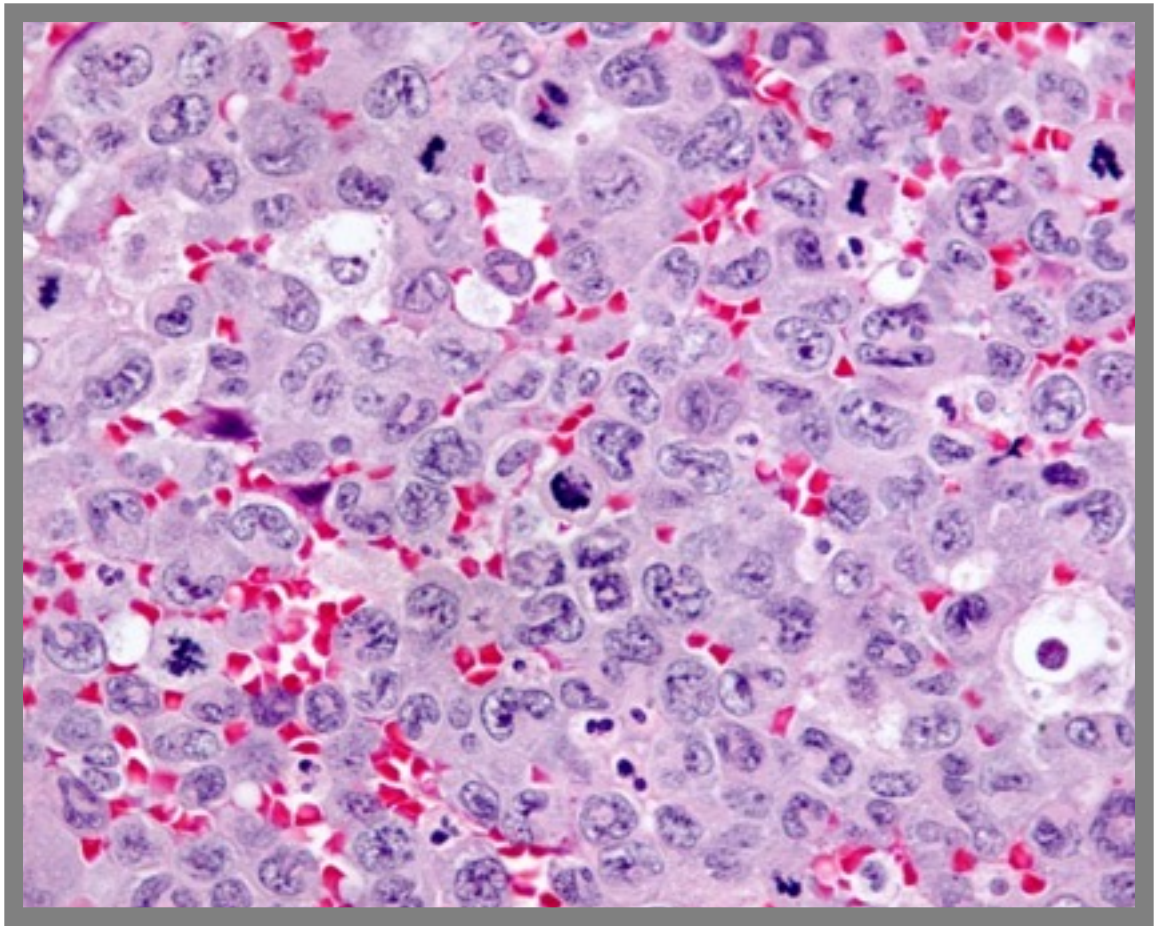
Small cells



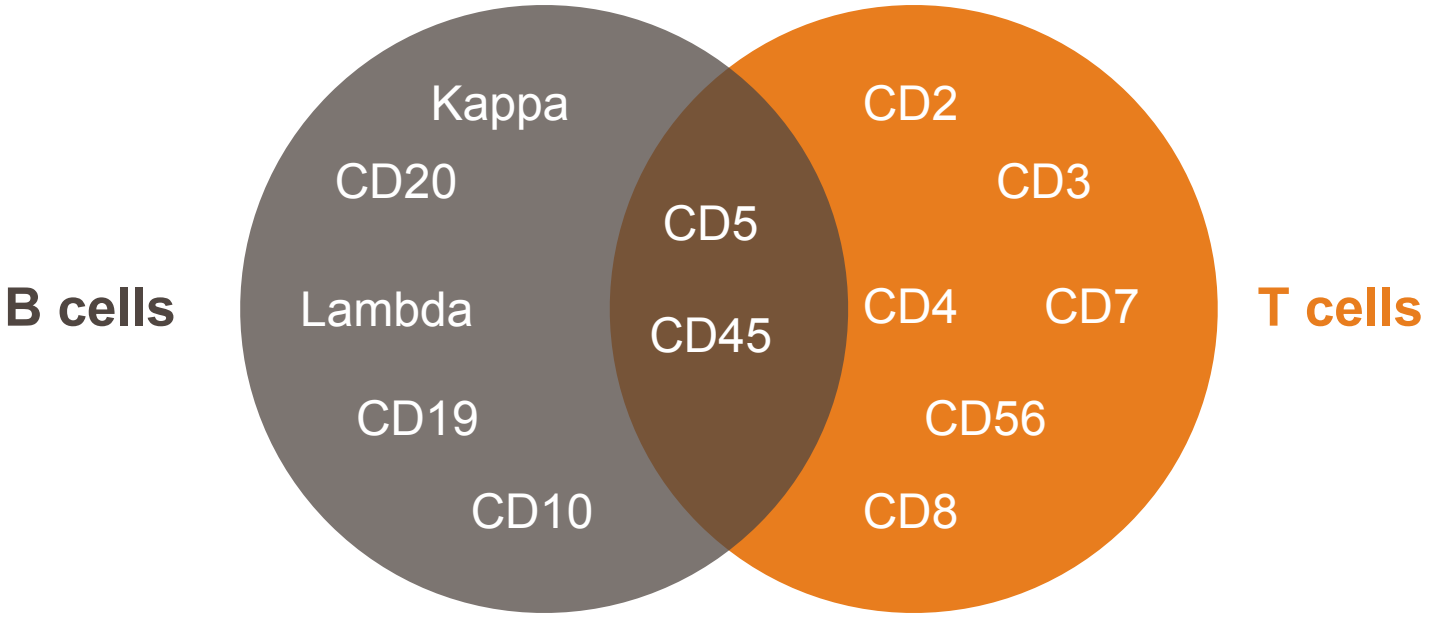
Large cells



Case study: morphology
What is the likely diagnosis?



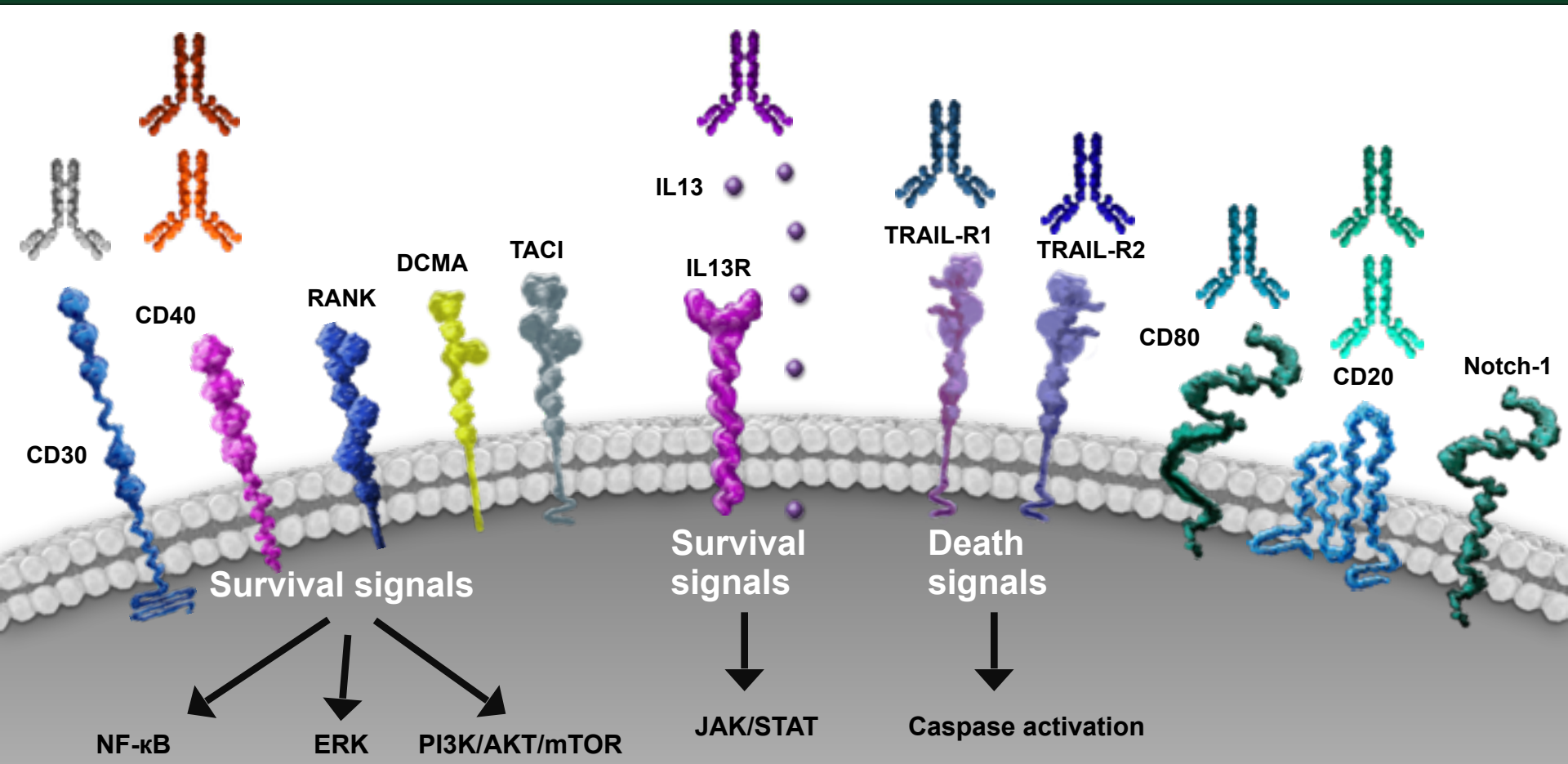
Immunophenotyping lymphomas involves multiple cell surface antigens[1,2]



Which markers would be part of your initial analysis?

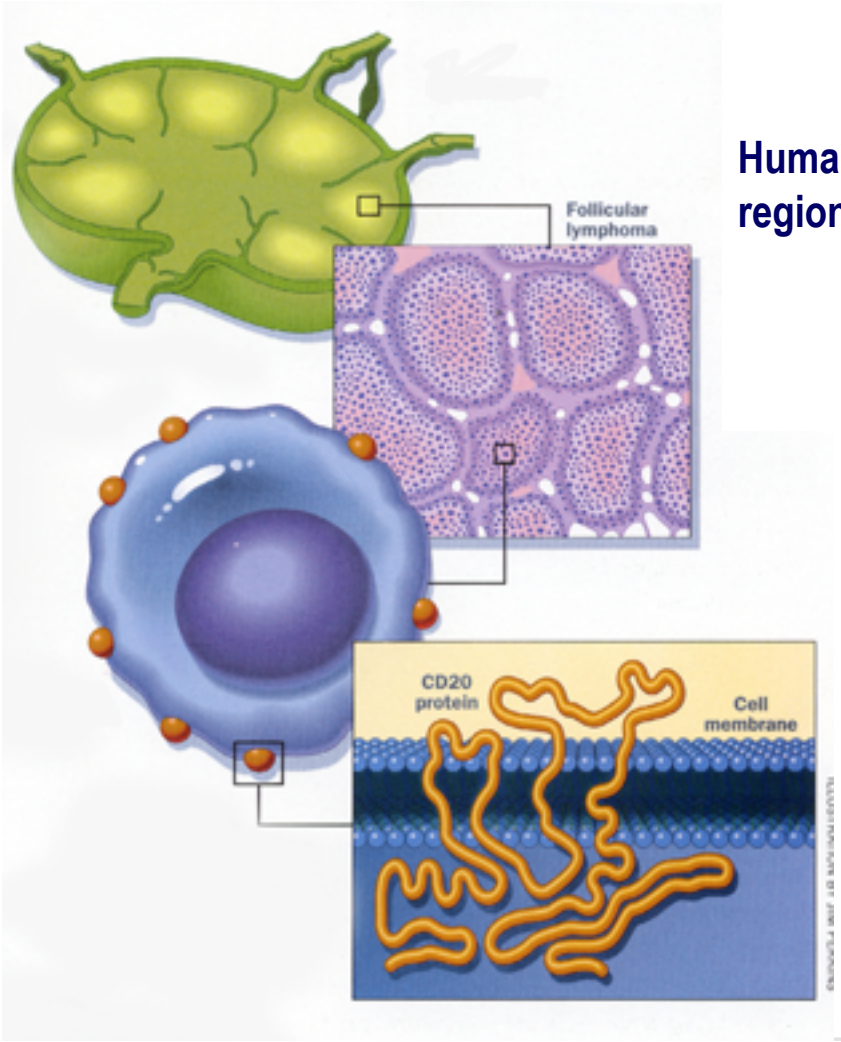
1. Harris NL et al. *Hematology Am Soc Hematol Educ Program.* 2001:194-220.
2. Wood BL et al. *Cytometry B Clin Cytom.* 2007;72(suppl 1):S14-S22.

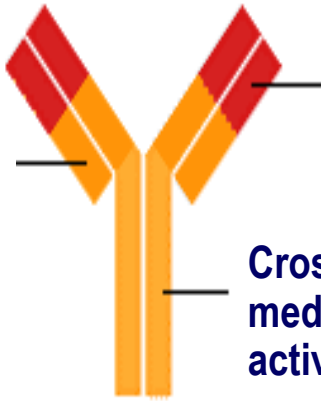
Targeting lymphoma with monoclonal antibodies:
multiple receptors and antigens that can be targeted



Younes A. *Nat Rev Clin Oncol*. 2011;8(2):85-96.

Rituximab:The first targeted therapy for Non-Hodgkin's Lymphoma

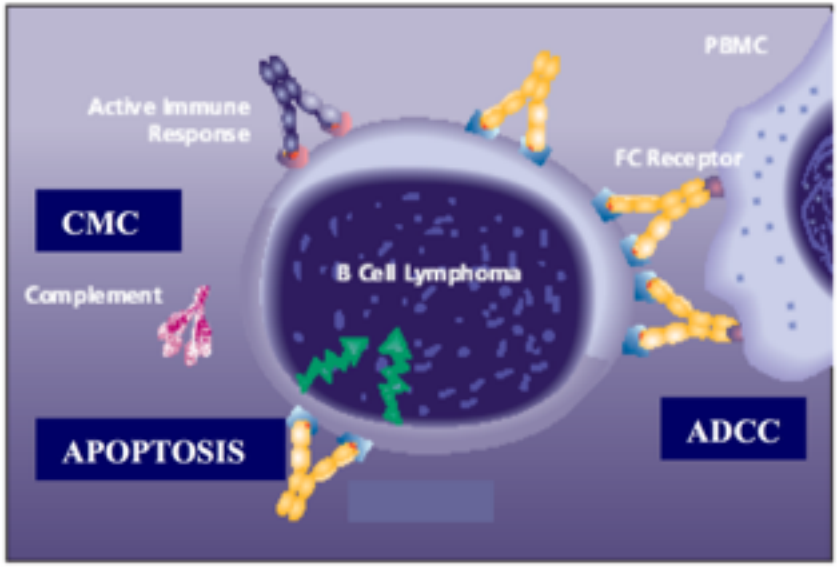




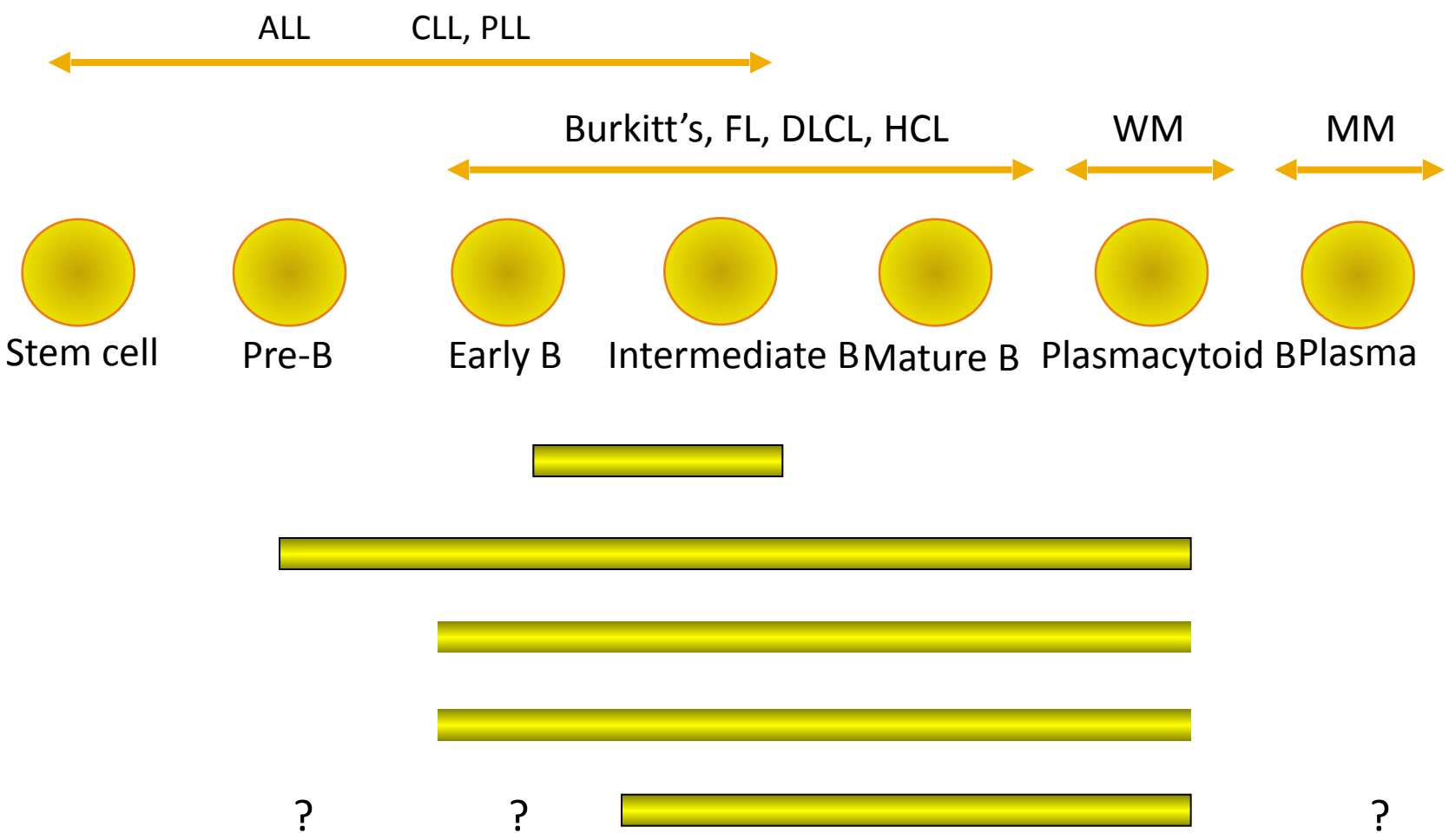
Human κ constant regions

Fab binds CD20 antigen present in B-cells

Crosslinking of the Fc portion mediates rituximab antitumor activity



Antigen Expression in B-Cell Lineage

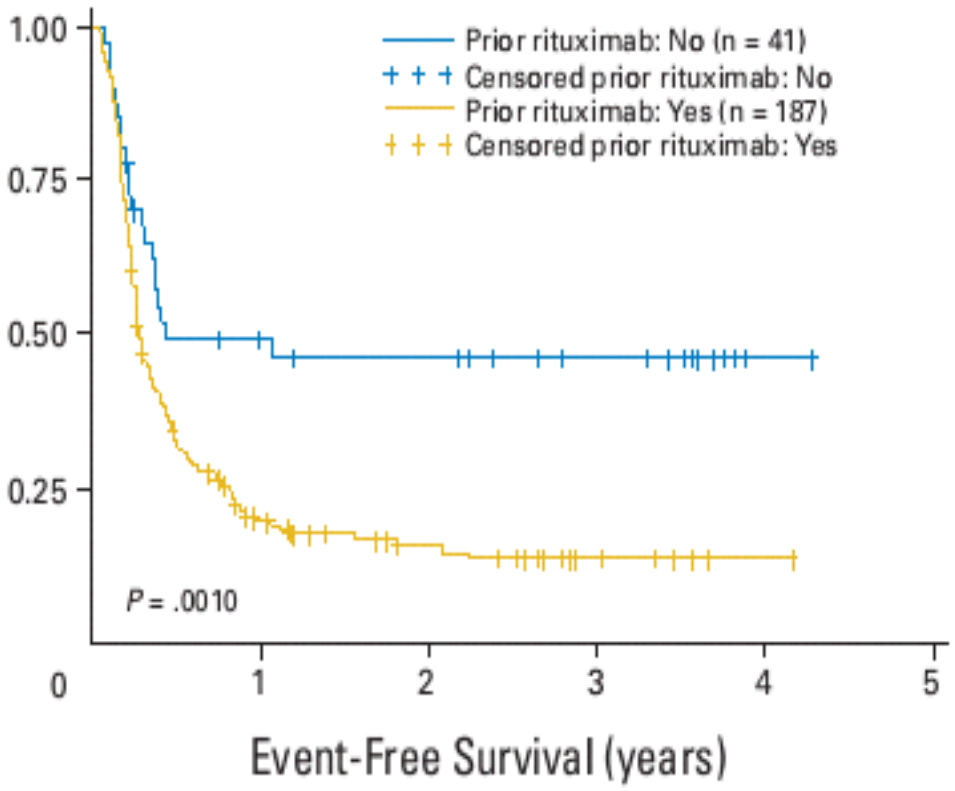


Randomized studies evaluating rituximab-chemotherapy in diffuse large B-cell lymphoma

Study	Phase	Patient population	Treatment arm (Number of patients per arm)	Response rate (%)	PFS (%)	OS (%)
Randomized studies defining the role of rituximab-chemotherapy in the management of DLBCL						
GELA (Coiffier et al, 2002)	III	Elderly patients with DLBCL (N=399)	R-CHOPx8 (202) vs. CHOPx8 (197)	83 vs. 69 (P=0.05)	At 5 years 57 vs. 38 (P<0.001)	At 5 years 70 vs. 57 (P=0.007)
MInT (Pfreundschuh et al, 2006)	III	Young patients with untreated DLBCL stage I bulky or II-IV, (N=823)	R-CHOPx6 (413) vs. CHOPx6 (410)	86 vs. 68 (P<0.01)	At 34 months 85 vs. 68 (P<0.0001)	At 34 months 93 vs. 84 (P=0.0001)
RICOVER-60 (Pfreundschuh et al,2008)	III	Elderly patients with untreated DLBCL (N=1222)	CHOPx6-14 (307) CHOPx8-14 (305) RCHOPx6-14 (306) RCHOPx8-14 (304)	68 vs. 72 (P=0.31) 78 (P=0.007) 76 (P=0.037)	At 3 years 56.9 vs. 56.9 (P=0.615) 73.4 (P<0.0001) 68.8 (P<0.0001)	At 3 years 67.7 vs. 66 (P=0.835) 78.1 (P=0.018) 72.5 (P=0.26)
ECOG 4494 (Morrison et al,2006)	III	Elderly patients with untreated DLBCL (N=632)	CHOPx6 (279) R-CHOPx6 (267) Responders were then randomized to RM or observation	77% vs. 76% before second randomization	At 3 years, FFS 39 vs. 52 (P=0.03) *After excluding RM patients	At 3 years, 57 vs. 67 (P=0.05) *After excluding RM patients

Prognostic Impact of Germinal Center B-cell (GCB)/ Activated B-Cell (ABC) Classification Analysed by Immunochemistry, FISH Analysis and GEP, In R/R DLBCL: The Bio-CORAL Study

Overall Response Rate	%	*P* Value
Total population	63%	-
CR/CRu	38%	
R-ICE	63.5%	-
R-DHAP	63%	
No Prior Rituximab	83%	< .0001
Prior Rituximab	51%	
Relapsed > 12 months	88%	< .0001
Refractory < 12 months	46%	
sIPI 0-1	71%	< .0002
sIPI 2-3	52%	



Thieblemont C. et al. J Clin Oncol. 2011;29:4079-87,

Strategies tested to improve the clinical outcome of DLBCL patients

Modality investigated	Improvement in response rate	Improvement in PFS or OS
Dose Dense R-CHOP14 vs. R-CHOP-21 LNH03-6B GELA study	No	No
Increase number of cycles R-CHOP x 6 vs. R-CHOP x 8 (RICOVER study)	No	No
Adding chemotherapy agents: R-CHOP vs. R-DA-EPOCH (Intergroup study CALGB50303/ECOG/SWOG)	Unknown	Unknown
High dose chemotherapy and autologous stem cell support (HDC0ASCS) in first remission for high risk DLBCL (Stiff et al., JCO 2011, #8011)	No	Favor in PFS at 2-years (69% vs. 56%, P=0.005). Study included CHOP and R-CHOP treated patients
Increasing intensity regimen without HDC-ASCT R-CHOP vs. R-Mega-CHOP	No	No
Rituximab Maintenance (ECOG 4494 and CORAL studies)	No	No

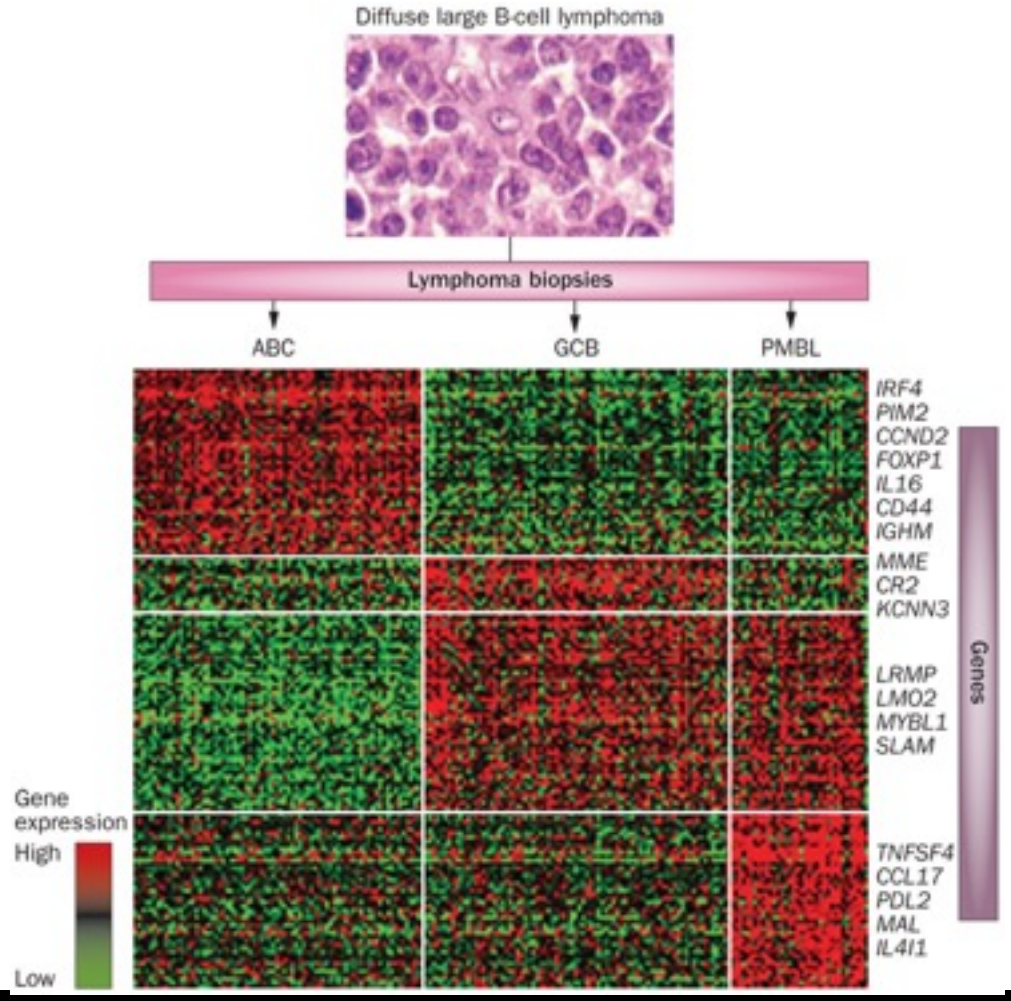
Current areas of research in aggressive B-cell lymphoma

1) Predicting patients that are less likely to respond to rituximab-CHOP in the front-line setting

2) The identification of key-regulatory pathways present in relapsed/refractory DLBCL

3) Can the targeting those pathways translate into clinical benefit?

Gene-expression profiling subdivides morphologically indistinguishable DLBCL tumors into three distinct cell-of-origin (COO) subtypes



PFS and overall survival for each DLBCL molecular subtype

Table 2 | PFS and overall survival for each DLBCL molecular subtype

Molecular subtype	Regimen	3-year PFS rate	3-year overall survival rate	Reference
ABC DLBCL	R-CHOP	40%	Approximately 45%	Lenz *et al.* (2008)[29]
GCB DLBCL	R-CHOP	74%	Approximately 80%	Lenz *et al.* (2008)[29]
PMBL	DA-EPOCH-R	100%*	97%*	Dunleavy *et al.* (2013)[16]

*At 5 years. Abbreviations: ABC, activated B-cell; DA-EPOCH-R, dose-adjusted etoposide, doxorubicin and cyclophosphamide with vincristine, prednisone and rituximab; DLBCL, diffuse large B-cell lymphoma; GCB, germinal centre B-cell; PFS, progression-free survival; PMBL, primary mediastinal B-cell lymphoma; R-CHOP, rituximab, cylophosphamide, doxorubicin, vincristine and prednisone.

Roschewski, M. et al. Nat. Rev. Clin. Oncol.2014; 11:11-22

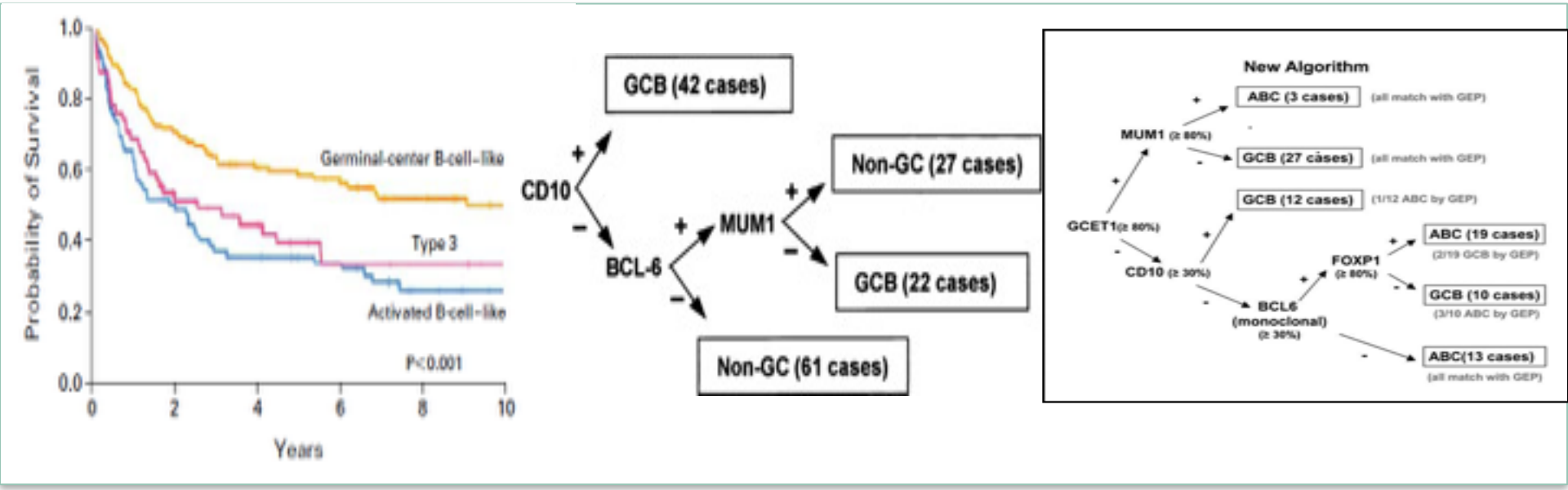
Oncogenic mechanisms and potential targets in DLBCL subtypes

Table 1 | Oncogenic mechanisms and potential targets in DLBCL subtypes

DLBCL subtype	Cell of origin	Oncogenic mechanisms	Potential targets
GCB	Germinal centre B-cell	*BCL2* translocation*	BCL6
		EZH2 mutations‡	EZH2
		PTEN deletions§	PI3K/Akt
		Loss of PTEN expression	
ABC	Post-germinal centre B-cell	NF-κB activation‖	BCR
		CARD11 mutations	CBM complex
		MYD88 mutations	IRAK-4
		CD79B mutations	JAK–STAT
		A20 deletions	
PMBL	Post-thymic B-cell	NF-κB activation¶	JAK–STAT
		9p24 amplification¶	PD-1#
		REL amplification	
		JAK2 mutations	
		CIITA translocations#	

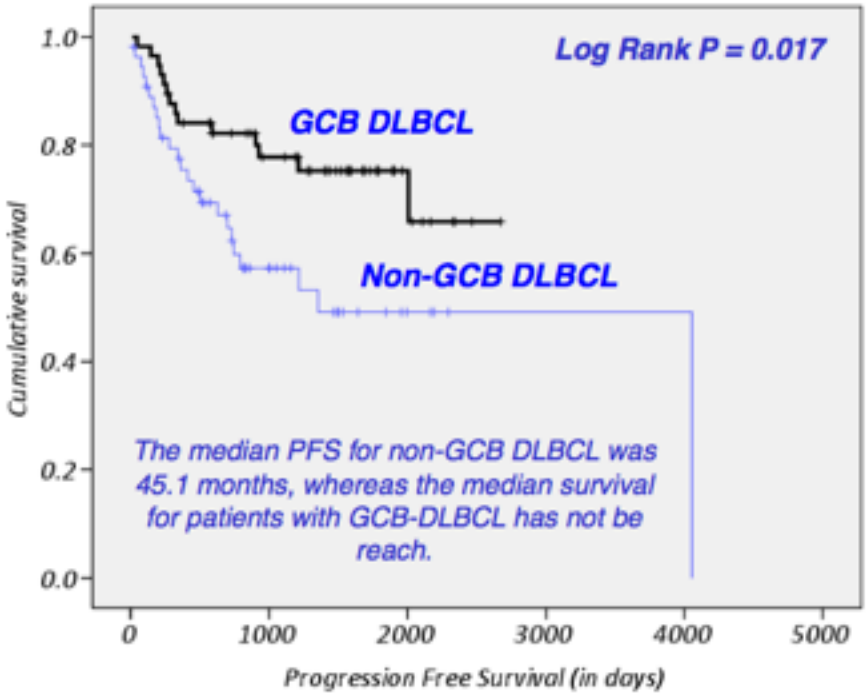
Roschewski, M. et al. Nat. Rev. Clin. Oncol.2014; 11:11-22

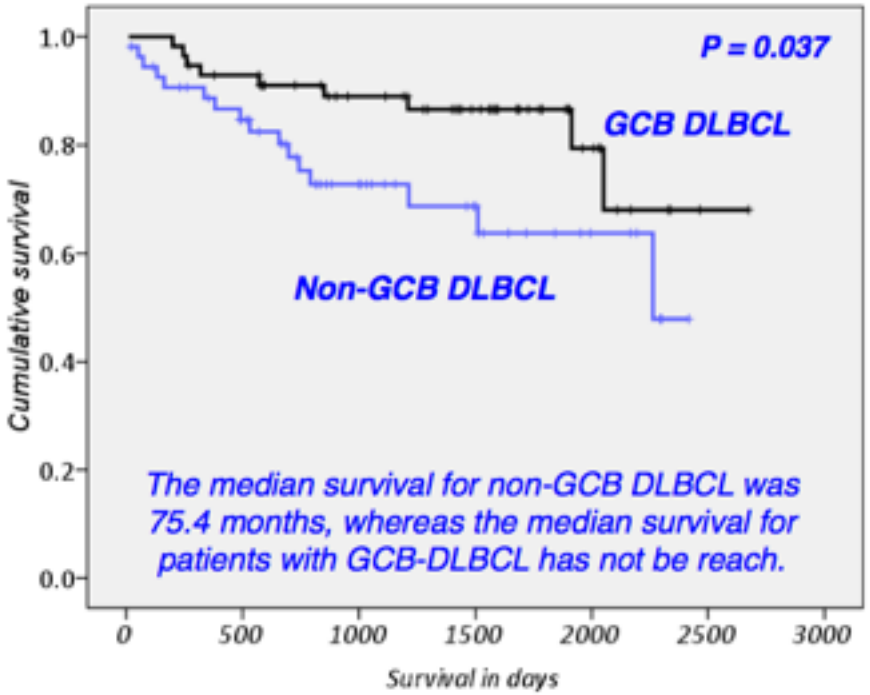
Molecular Profiling in DLBCL



Rosenwald et al., NEJM 2002
Hans et al., Blood 2004
Choi et al., Clin Cancer Res 2009

DLBCL with a non-GCB phenotype by IHC had an inferior PFS and OS following R-CHOP-21 than GCB-DLBCL



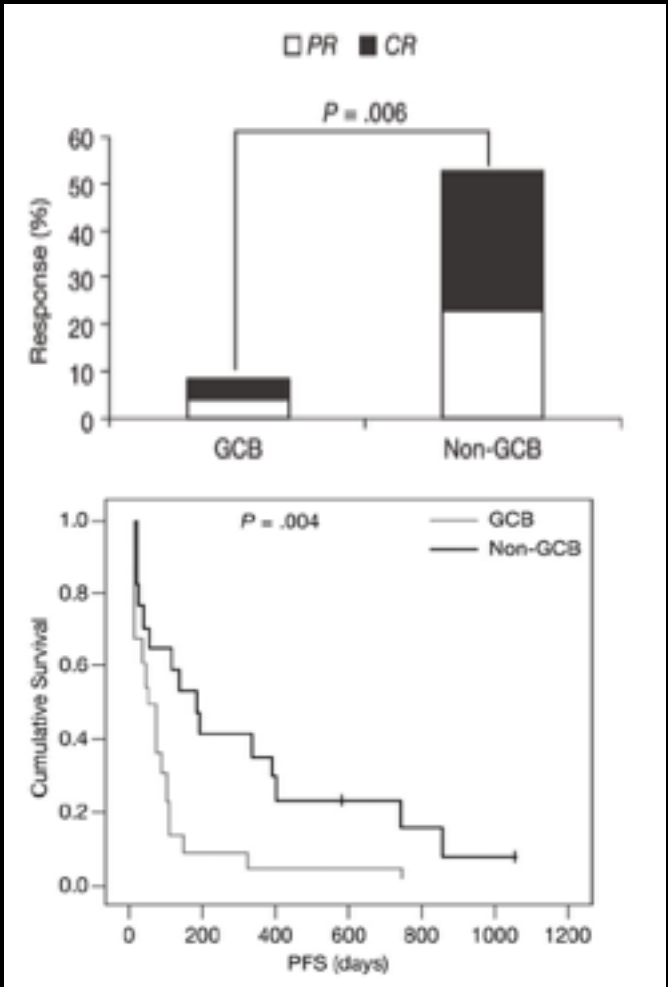


Chavez et al. ASH 2009: Abstract 623

New strategies to improve clinical outcome

Target the "bad lymphomas" ? - *Should we treat based on COO or IHC results. If so with what?*

Targeting the cell of origin

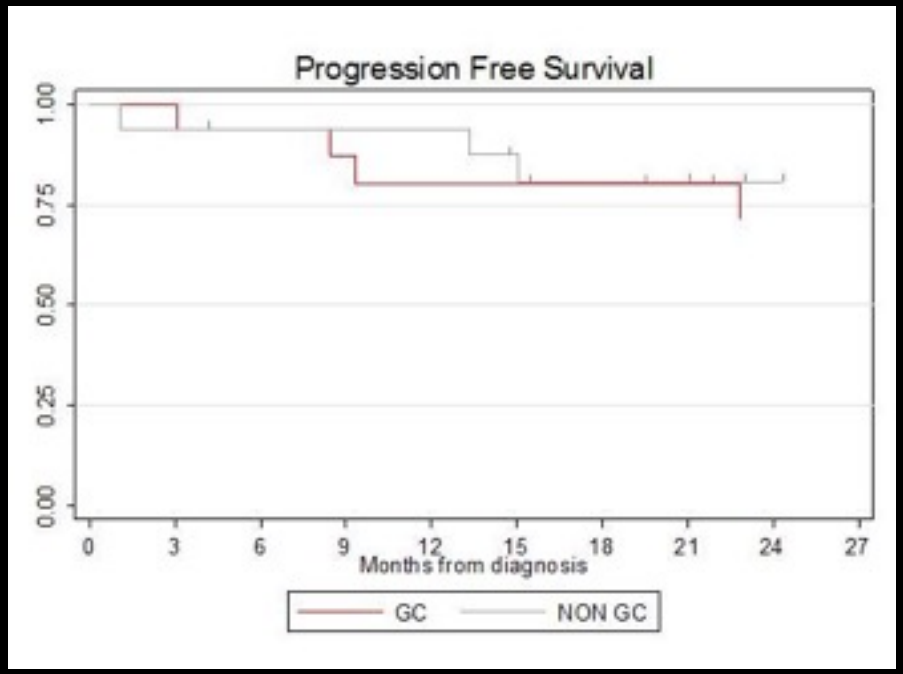


- Non-GCB phenotype had a higher ORR as compared with GCB

- Ongoing phase II study prospectively randomizing patients based on COO

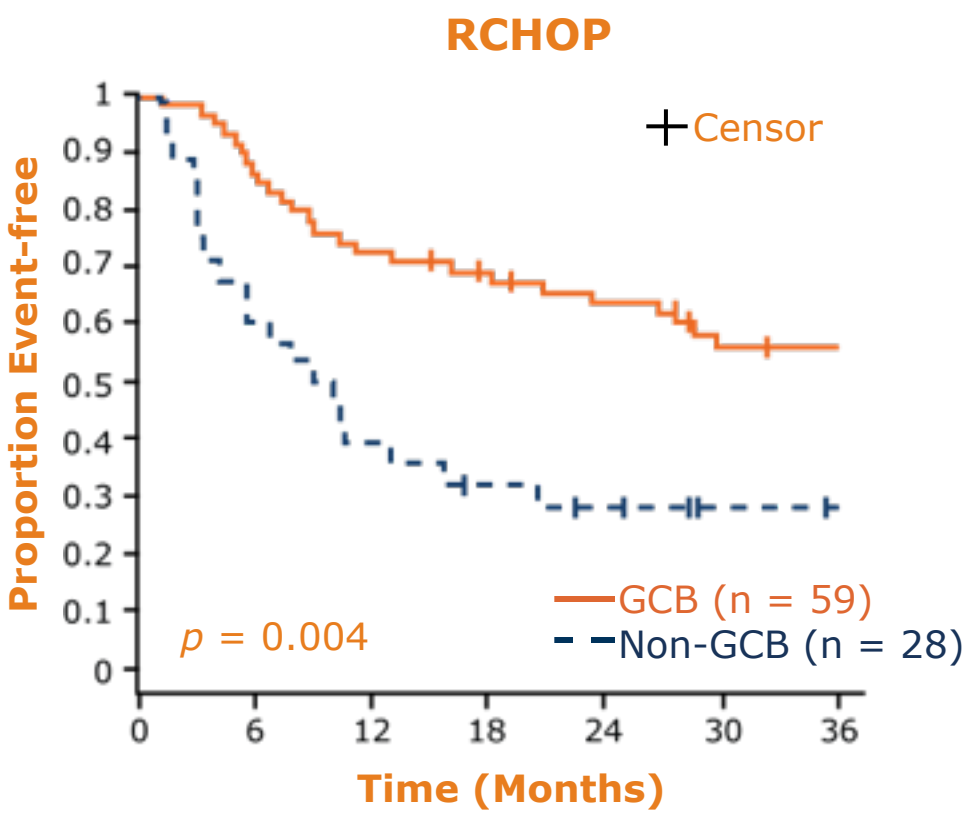
Final Results of Phase II Study of Lenalidomide Plus Rituximab-CHOP21 in Elderly Untreated DLBCL Focusing on Cell of Origin: REAL07 Trial of the Fondazione Italiana Linfomi

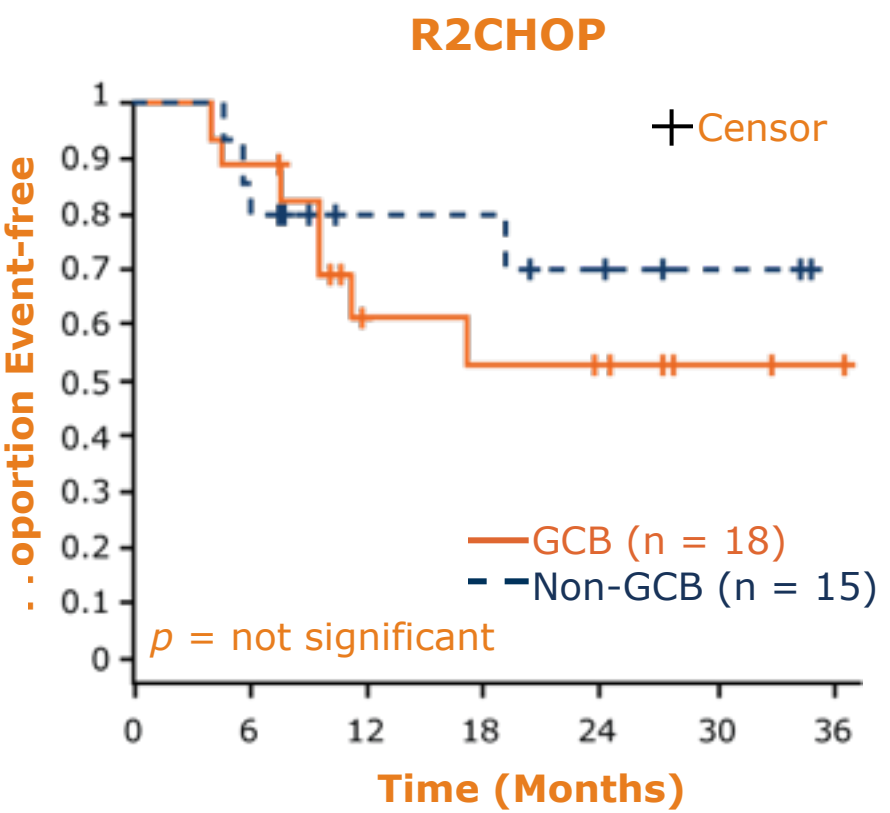
	ORR	CR	PR	2-yr OS	2-yr PFS
Overall (49)	92%	86%	6%	92%	80%
GCB (16)	88%	81%			71%
non-GCB (16)	88%	88%			81%



Chiappella A et al. ASH 2013 Abstract 850

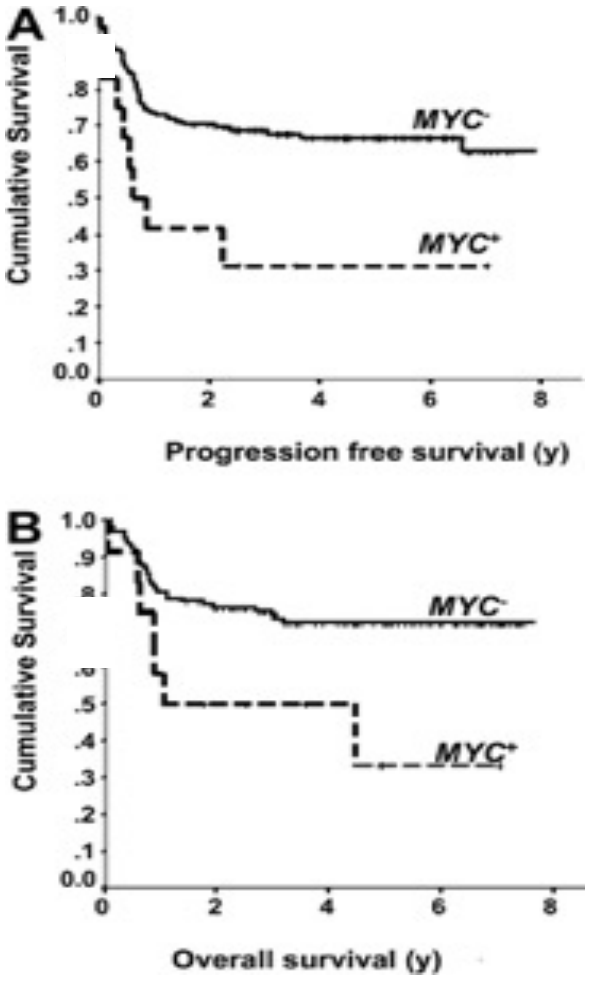
PFS by GCB versus Non-GCB Subtype with R2CHOP versus R-CHOP





Nowakowski GS al. Proc ASH 2012;Abstract 689.

c-MYC expression in DLBCL



- **9% of newly diagnosed DLBCL appear to harbor the myc translocation**
- **Myc+ DLBCL have an overall poor prognosis when treated with R-CHOP**
- **Ki-67 score cannot identify patients at high risk of harboring the MYC rearrangement**
- **Patients with MYC+ DLBCL should be treated with aggressive regimens or referred a clinical trial**

Savage K et al., Blood 2009

Impact of Induction Regimen and Stem Cell Transplantation on Outcomes in Patients with Double Hit Lymphoma: A Large Multicenter Retrospective Analysis

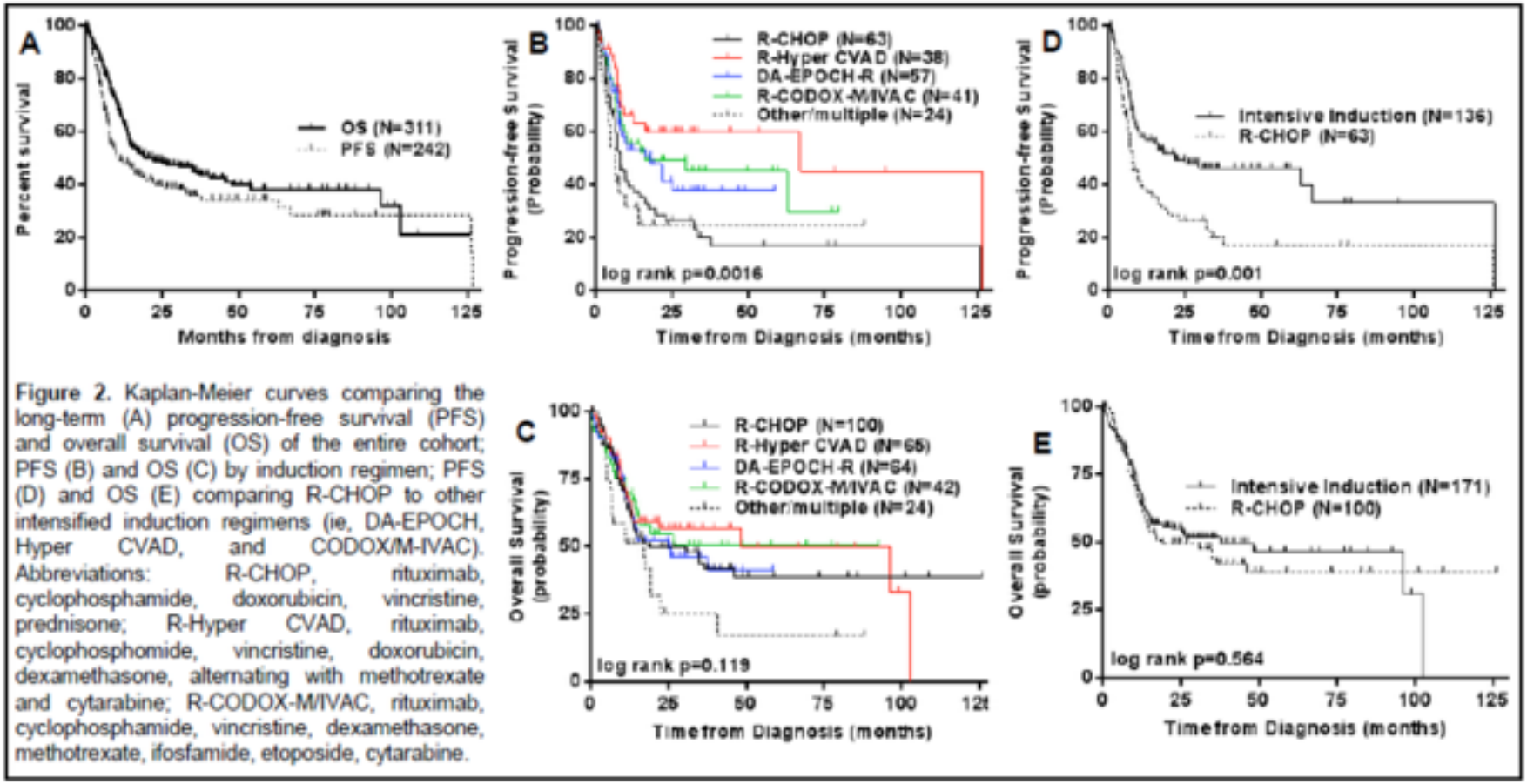


Figure 2. Kaplan-Meier curves comparing the long-term (A) progression-free survival (PFS) and overall survival (OS) of the entire cohort; PFS (B) and OS (C) by induction regimen; PFS (D) and OS (E) comparing R-CHOP to other intensified induction regimens (ie, DA-EPOCH, Hyper CVAD, and CODOX/M-IVAC). Abbreviations: R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; R-Hyper CVAD, rituximab, cyclophosphomide, vincristine, doxorubicin, dexamethasone, alternating with methotrexate and cytarabine; R-CODOX-M/IVAC, rituximab, cyclophosphamide, vincristine, dexamethasone, methotrexate, ifosfamide, etoposide, cytarabine.

Gandhi M, et al, Blood 2013 122:640

Impact of Induction Regimen and Stem Cell Transplantation on Outcomes in Patients with Double Hit Lymphoma: A Large Multicenter Retrospective Analysis

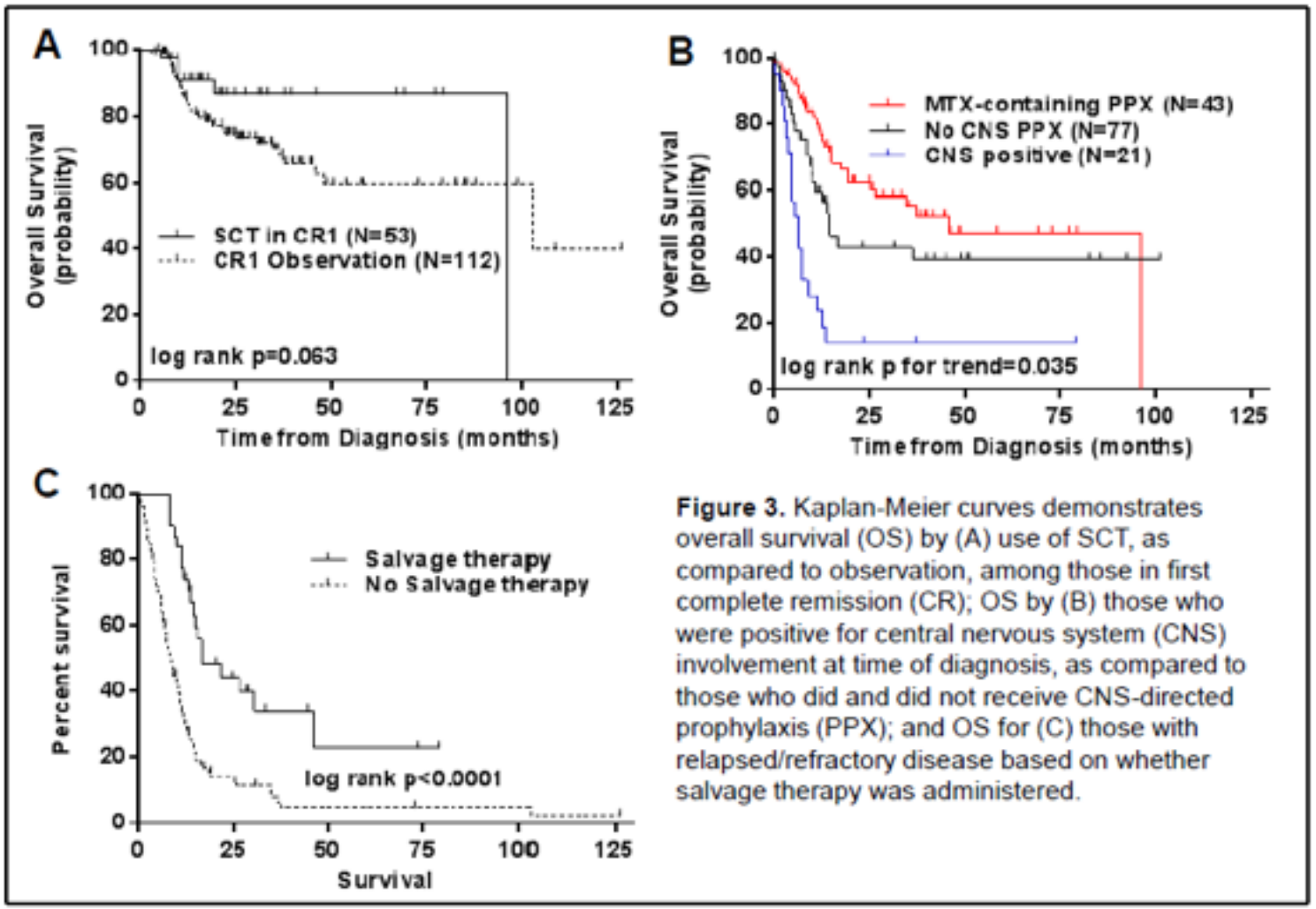


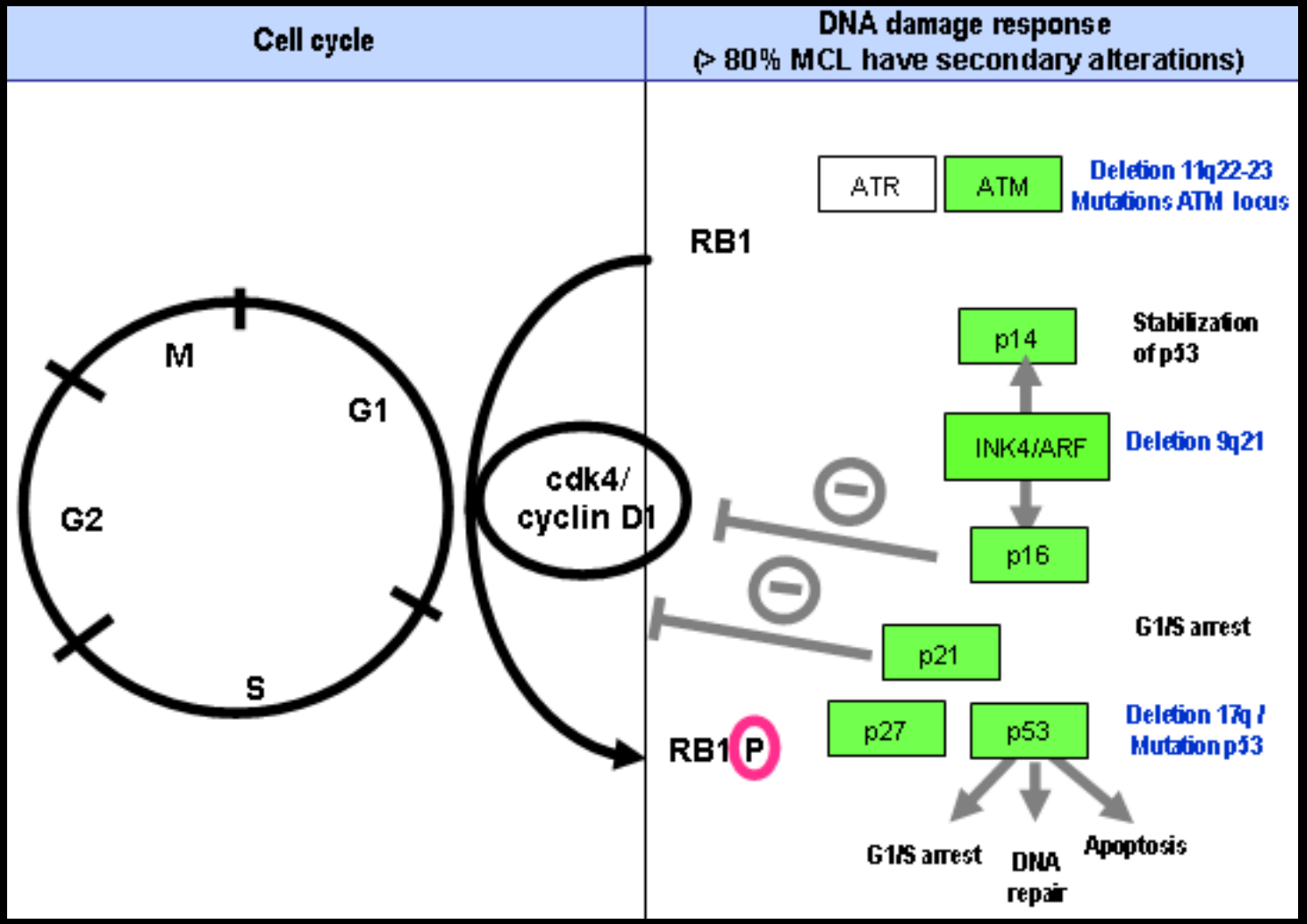
Figure 3. Kaplan-Meier curves demonstrates overall survival (OS) by (A) use of SCT, as compared to observation, among those in first complete remission (CR); OS by (B) those who were positive for central nervous system (CNS) involvement at time of diagnosis, as compared to those who did and did not receive CNS-directed prophylaxis (PPX); and OS for (C) those with relapsed/refractory disease based on whether salvage therapy was administered.

Gandhi M, et al, Blood 2013 122:640

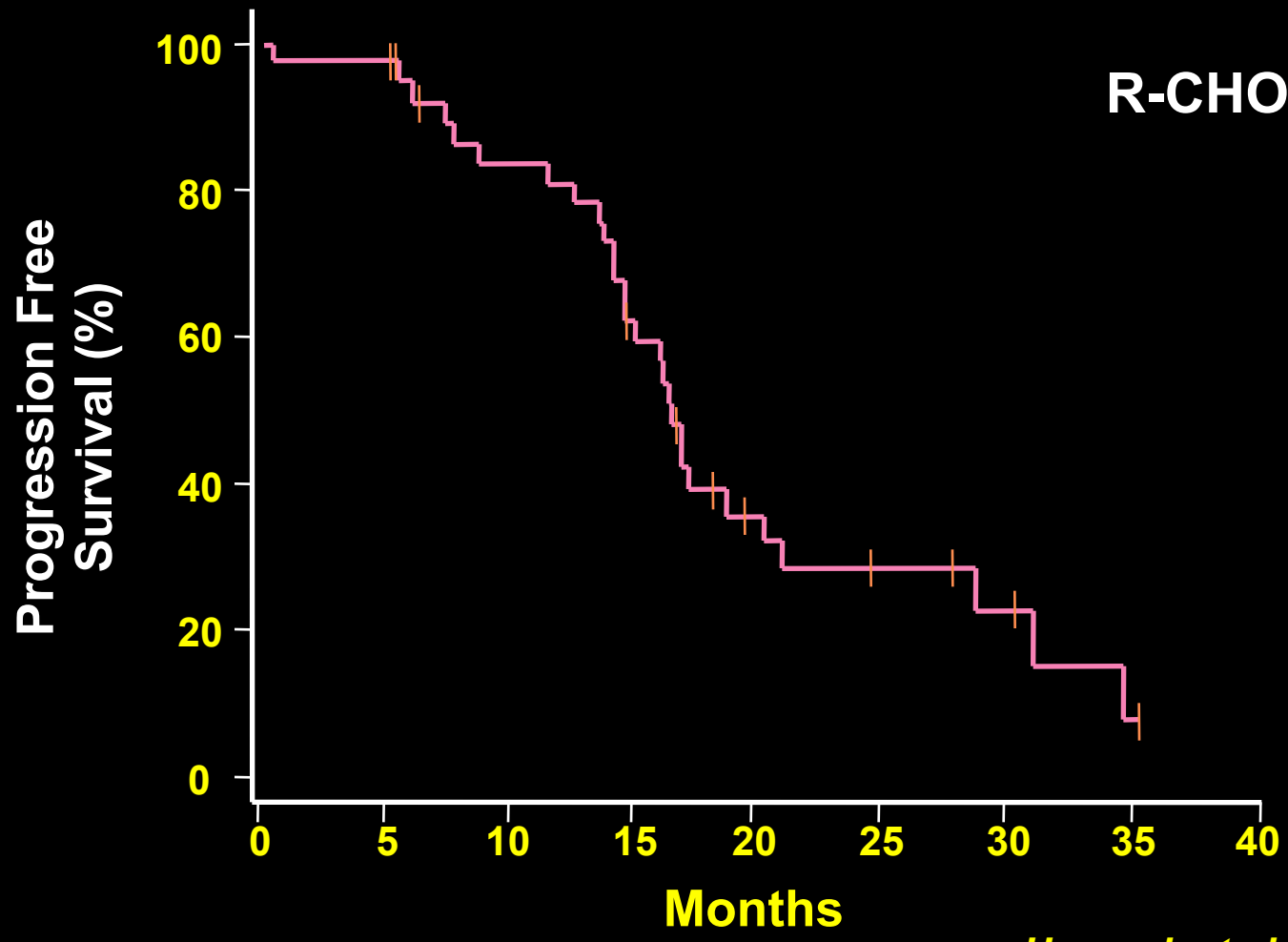
Mantle Cell Lymphoma

- Classified as unique entity in 1992
- Mantle cell lymphoma (MCL) comprises 5–10% of all non-Hodgkin's lymphomas. Approximately 2,000 cases per year in US
- Characteristic bcl-1 translocation = t(11;14)
- Mantle cell lymphoma (MCL) has an aggressive clinical course with a median survival < 3 years and is incurable with conventional chemotherapy.
  - CHOP: 40% CR rate, Median PFS of 1-2 yrs and OS of 3 yrs.
  - HDT with auto SCT = 2-4 year DFS of 30%
  - Rituximab alone* (n=81): ORR = 37% (14% CR), Median DR = 1.2 years

Vandenberghe, E et al. Br J Haematol, 2003; 120: 793–800

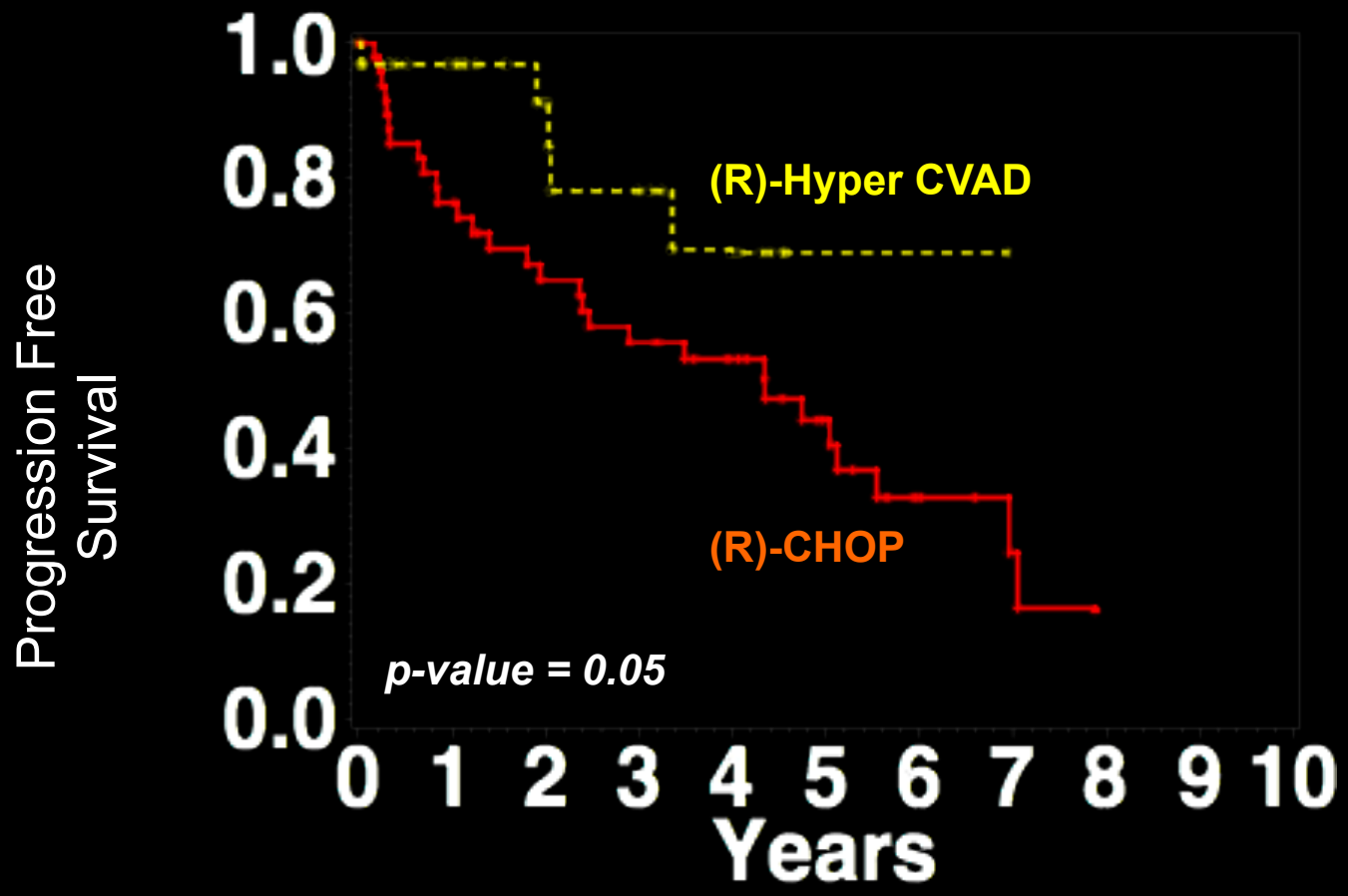

Cell cycle
DNA damage response
(> 80% MCL have secondary alterations)
M
G1
G2
S
cdk4/
cyclin D1
ATR
ATM
Deletion 11q22-23
Mutations ATM locus
RB1
p14
Stabilization
of p53
INK4/ARF
Deletion 9q21
p16
p21
G1/S arrest
RB1 P
p27
p53
Deletion 17q /
Mutation p53
G1/S arrest
DNA
repair
Apoptosis

Mantle Cell Lymphoma: Outcomes of patients treated with R-CHOP



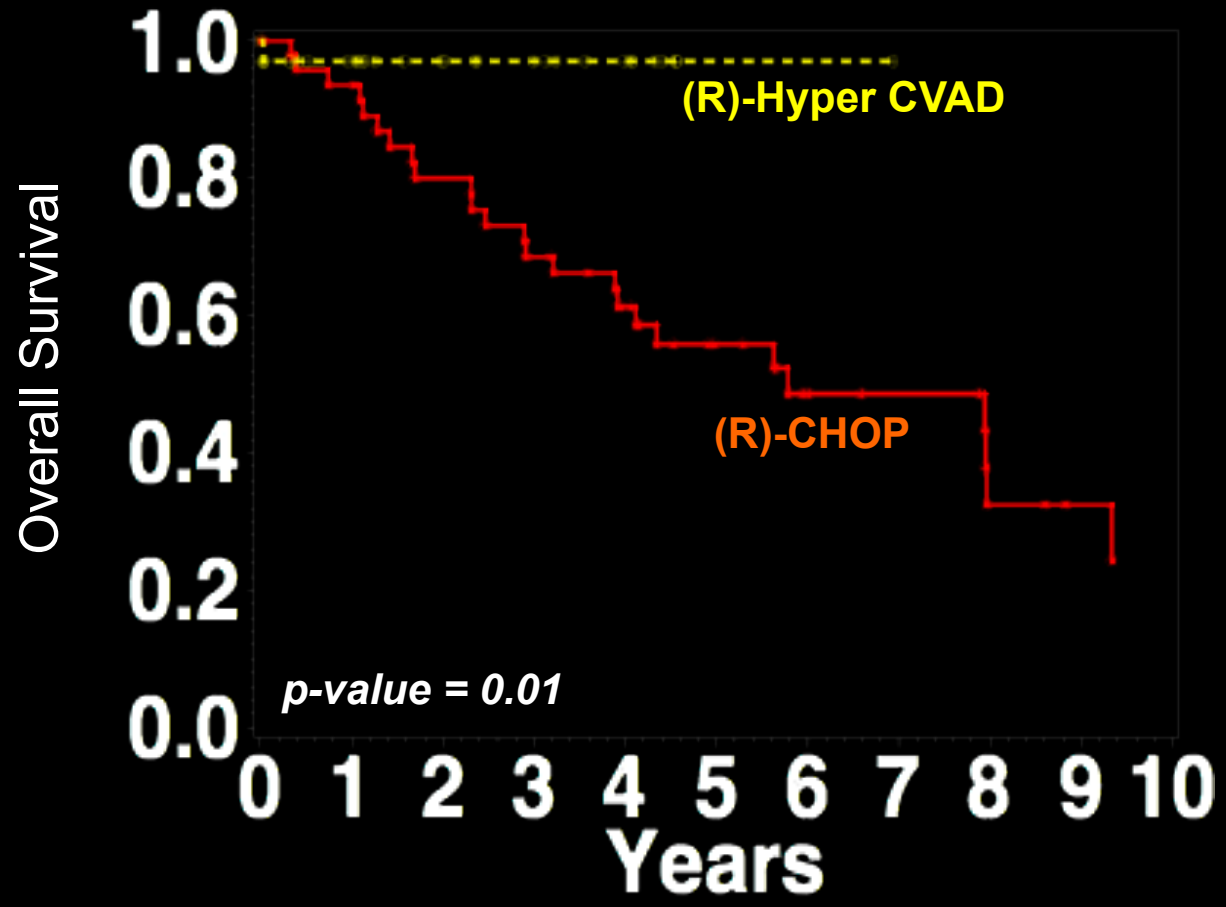
Howard, et al: JCO 20, 2002

Mantle Cell Lymphoma: R-CHOP vs R-Hyper CVAD



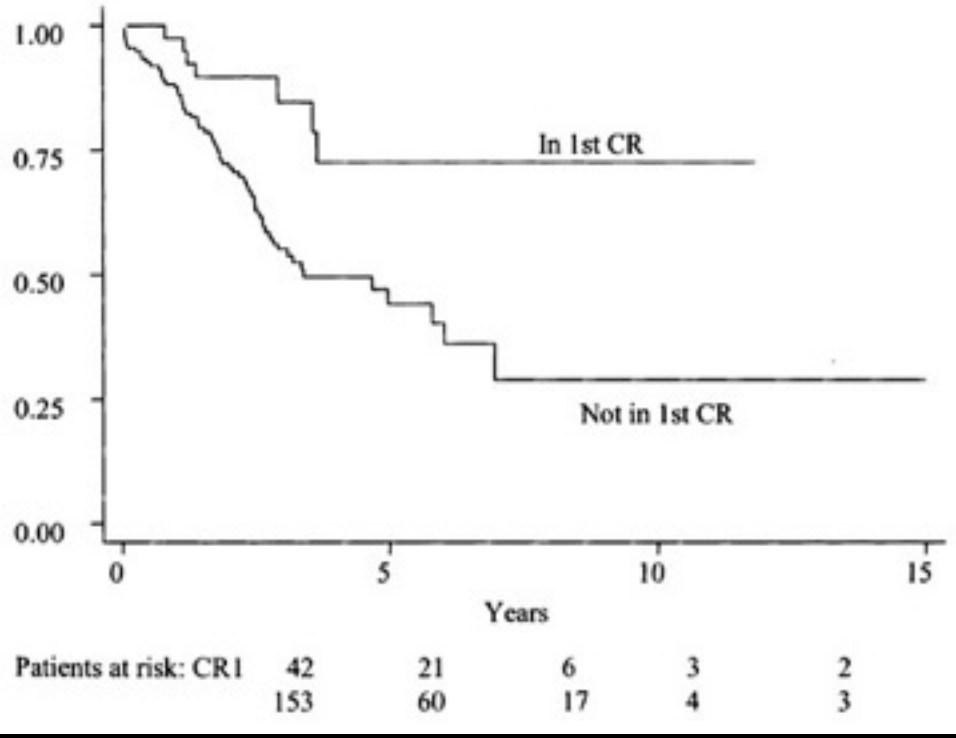
Vose et al, Procc Am Soc Clin Onc 2006; 7511a

Mantle Cell Lymphoma: R-CHOP vs R-Hyper CVAD

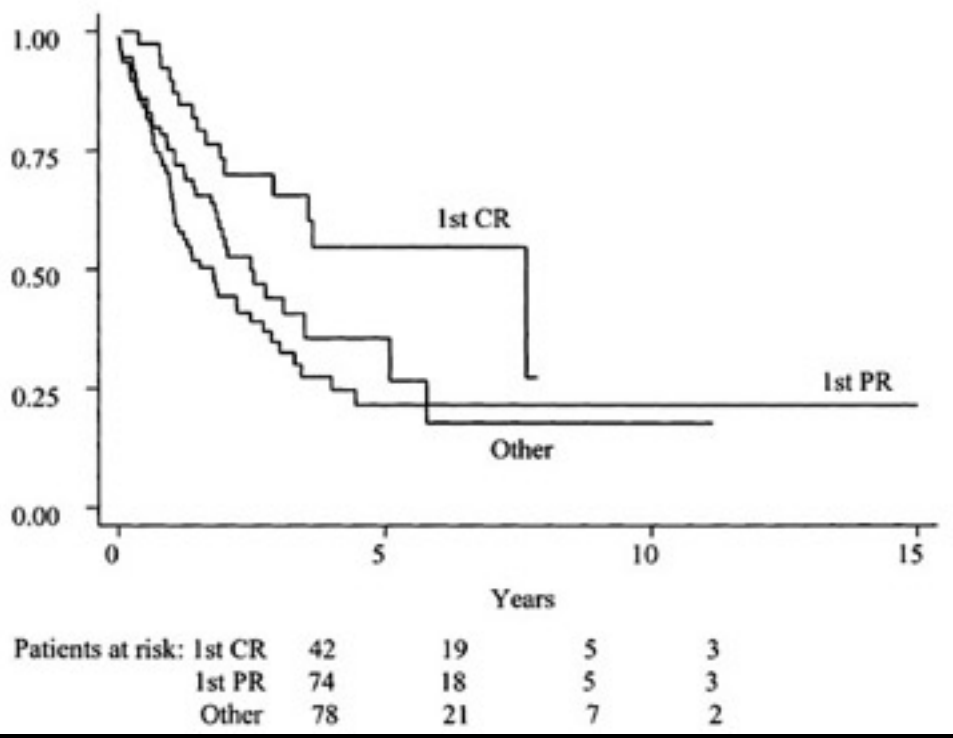


Vose et al, Procc Am Soc Clin Onc 2006; 7511a

Mantle Cell Lymphoma: a case for early transplantation



Overall survival from time of transplantation by disease status.



Progression-free survival from time of transplantation by disease status

Vandenberghe, E et al. Br J Haematol, 2003; 120: 793–800

T-cell lymphoma is a heterogeneous disease comprised of multiple subtypes[1,2]

Leukemic or disseminated

- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Chronic lymphoproliferative disorders of NK cells*
- Aggressive NK-cell leukemia
- Adult T-cell lymphoma/leukemia (HTLV1-pos
- Systemic E (EBV)–posi of childhoo

Cutaneous

- Mycosis fungoides
- Sézary syndrome
- Primary cutaneous CD30-positive lymphoproliferative disorders
- Primary cutaneous anaplastic large cell lymphoma
- Lymphomatoid papulosis

lymphoma
ma
rmotropic CD8-
D4-positive

Nodal

- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma, ALK-positive
- Anaplastic large cell lymphoma, ALK-negative*
- Peripheral T-cell lymphoma, not otherwise specified

Nodal

- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma, ALK-positive
- Anaplastic large cell lymphoma, ALK-negative
- Peripheral T-cell lymphoma, not otherwise specified

Extranodal

- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy-associated T-cell lymphoma
- Hepatosplenic T-cell lymphoma

*Provisional entity.

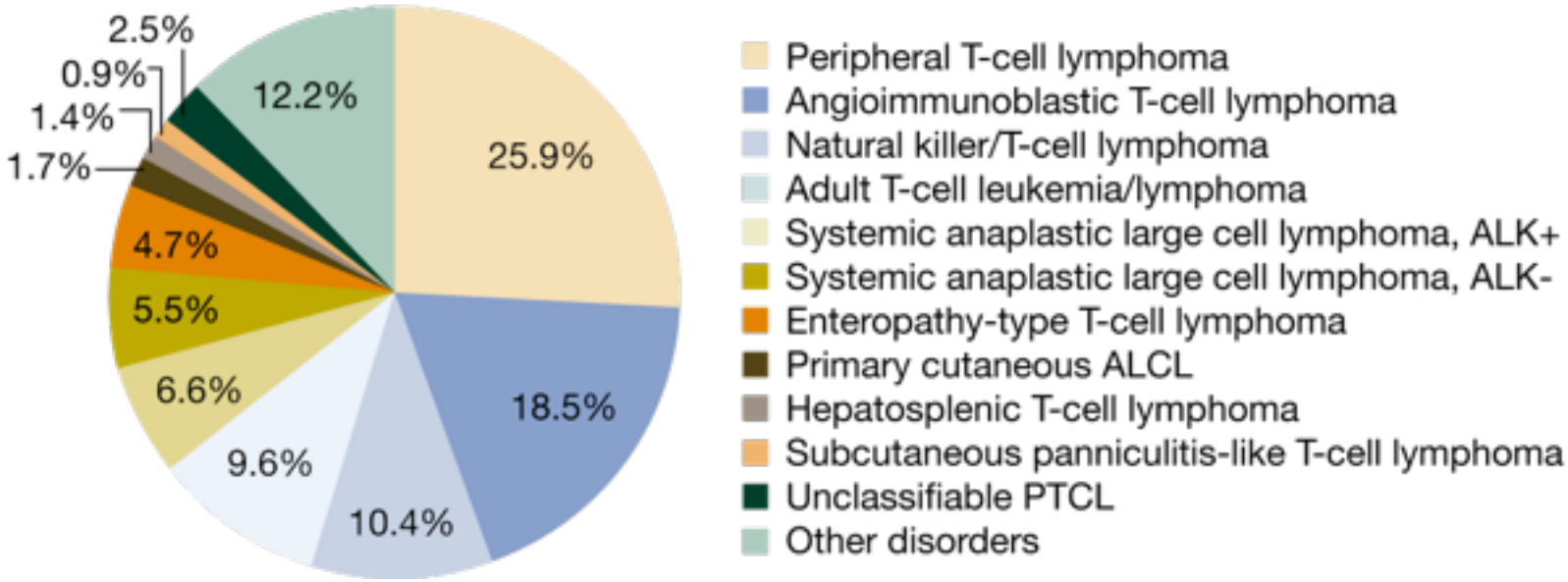
1. Swerdlow SH et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC; 2008.

2. de Leval L et al. *Hematology Am Soc Hematol Educ Program*. 2011:336-343.

International T-Cell Lymphoma Project: pathology findings and clinical outcomes[1,2]

- Goal: to evaluate the role of clinical data in T-cell lymphoma diagnosis
- 1,314 cases were reviewed by expert hematopathologists and classified according to WHO criteria

Relative frequencies of mature T-cell lymphomas



1. Vose JM et al. *J Clin Oncol.* 2008;26(25):4124-4130.
2. Swerdlow SH et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC; 2008.

International T-Cell Lymphoma Project: pathology findings and clinical outcomes (continued)

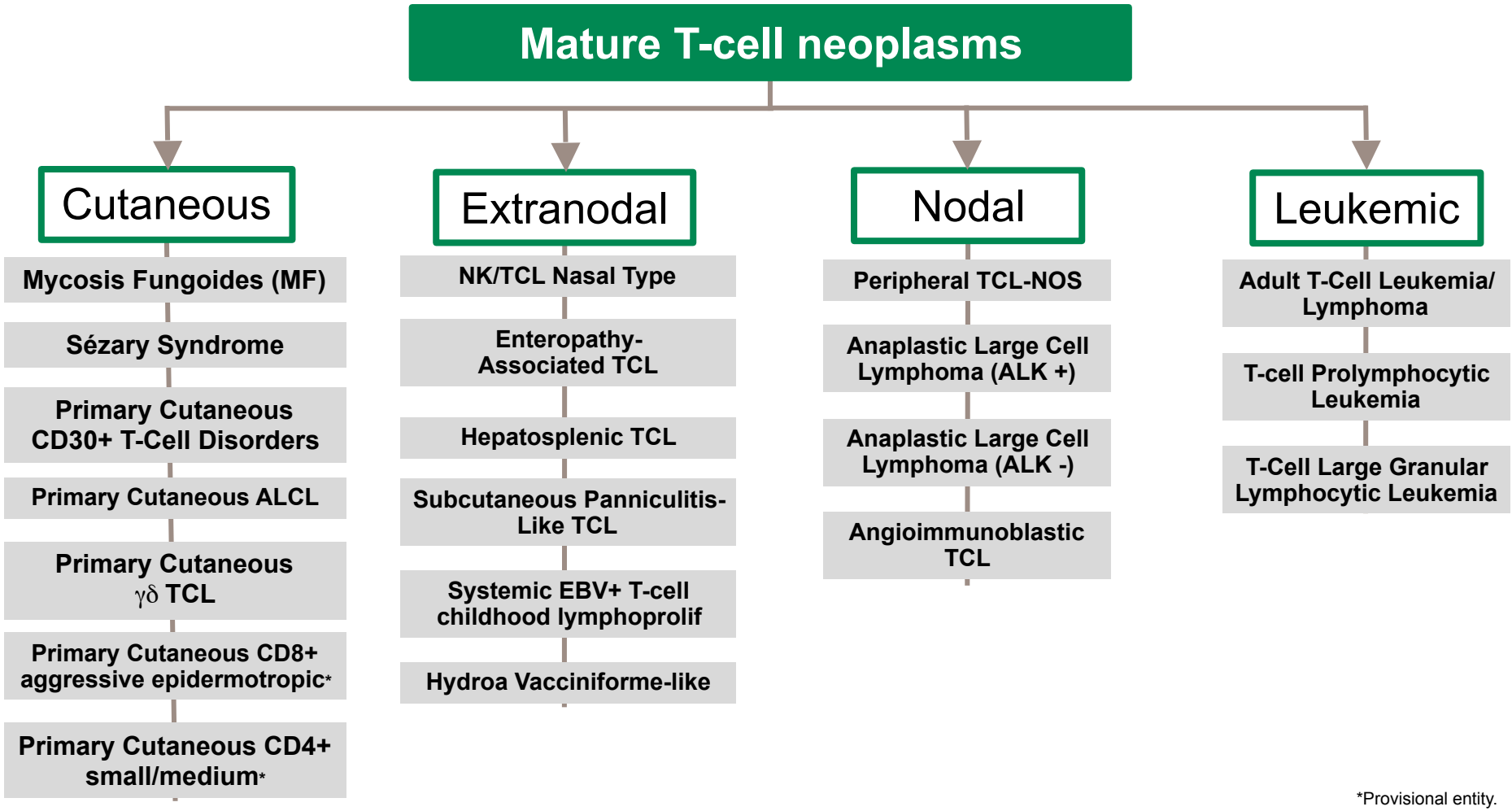
- Found that diagnosing distinct T-cell lymphoma subtypes can be challenging[1]
- Subsets with specific diagnostic markers have higher rates of diagnostic accuracy (agreement with consensus diagnosis)[2]

Good (>90% agreement)[2]	Generally poor (<85% agreement)[2]
• ALCL, ALK-positive: 97% • ATLL: 93% • NKTCL: 92%	• Primary cutaneous ALCL: 66% • Hepatosplenic: 72% • ALCL, ALK-negative: 74% • PTCL-NOS: 75% • Subcutaneous panniculitis-like: 75% • EATL: 79% • AITL: 81%

- The addition of clinical data can aid the diagnosis of certain lymphomas[1]:
  - When experts were provided with HTLV-1 status, 39% of PTCL-NOS cases were changed to ATLL
- 10.4% of lymphoma cases could not be classified or were misdiagnosed[2]

1. Armitage JO et al. *Clin Adv Hematol Oncol.* 2010;8(12)(suppl 22):1-15.
2. Vose JM et al. *J Clin Oncol.* 2008;26(25):4124-4130.

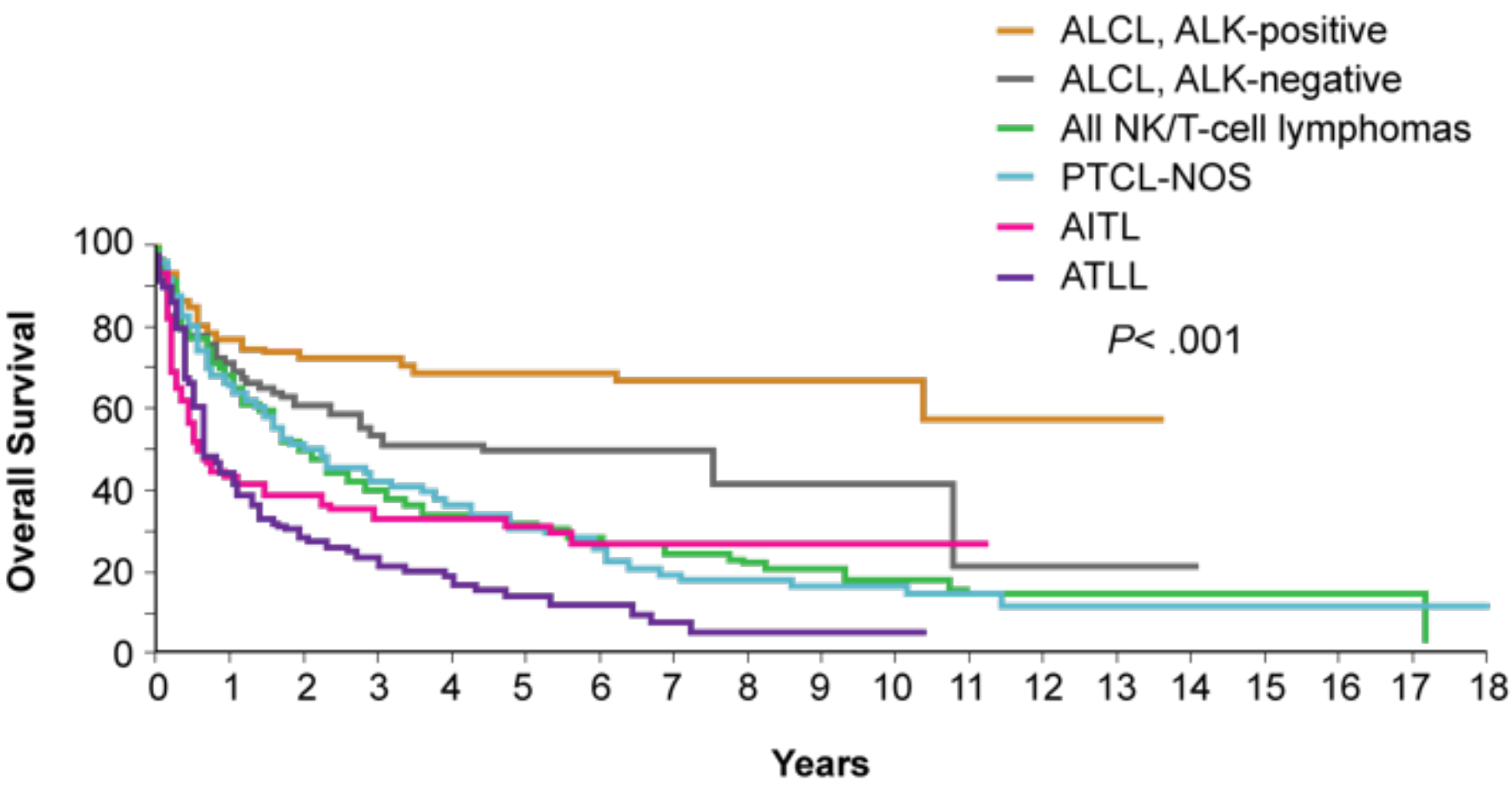
2008 World Health Organization classification of PTCL



*Provisional entity.

1. Swerdlow SH et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC; 2008.
2. de Leval L, Gaulard P. *Hematology Am Soc Hematol Educ Program*. 2011:336-343.

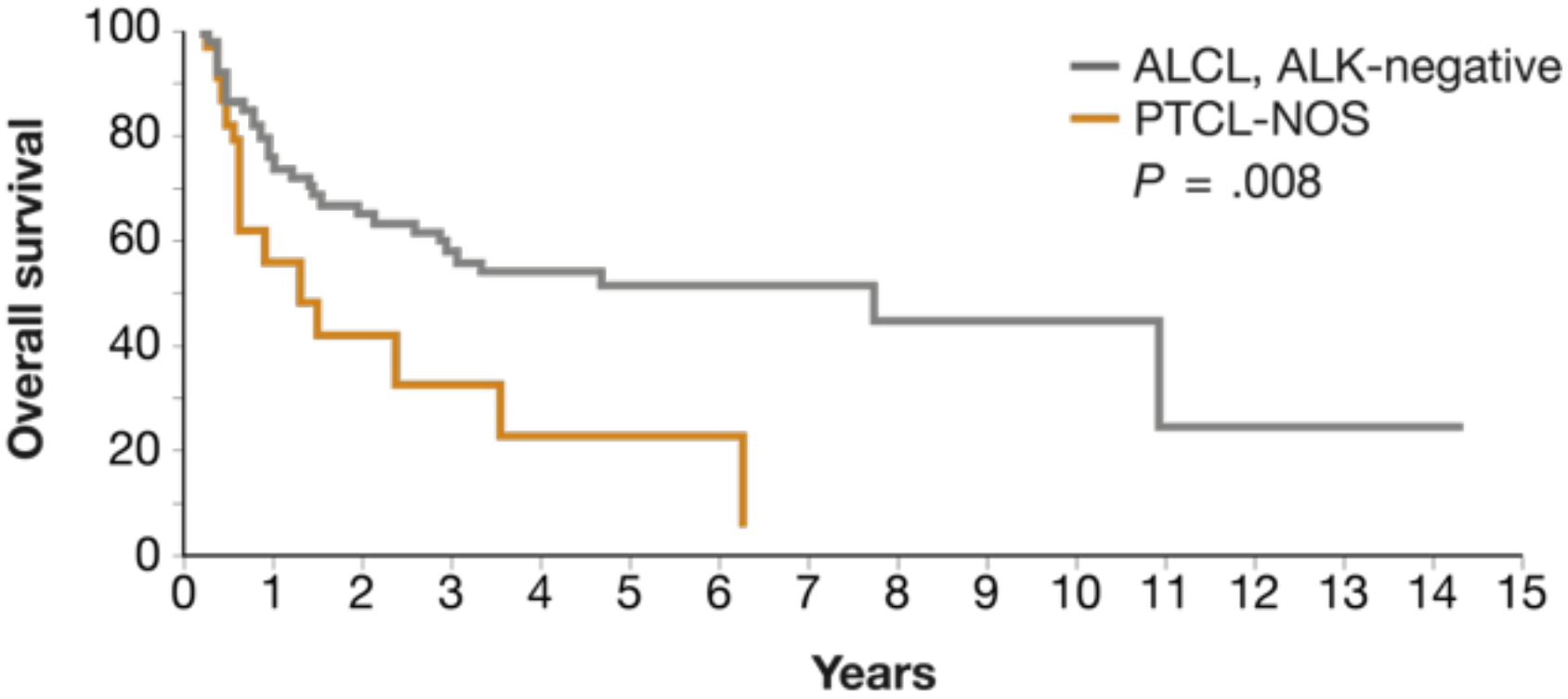
PTCL prognosis by subtype



International T-Cell Lymphoma Project. *J Clin Oncol.* 2008;26(25):4124-4130.

ALK-negative ALCL has a better prognosis than PTCL-NOS

5-year overall survival of ALK-negative ALCL and PTCL-NOS
(CD30+ ≥80% of cells)



Savage KJ et al. *Blood*. 2008;111(12):5496-5504.


ROSWELL PARK