Non Hodgkin Lymphoma: Immunological Markers & Newer Treatment

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- NHL
  - Comprises 2.7% of all cancers worldwide
  - Commonest hematological malignancy
  - Not a single disease but a heterogeneous group of neoplasms, > 60 types of NHLs

Diagnosis of NHL

- Clinical presentations are quite variable and non specific
- It's a heterogeneous group of malignancy in terms of
  - Cell of origin
  - Clinical course
  - Prognosis and
  - Response to therapy
- Proper tissue diagnosis including precise subtyping is the key to successful treatment of NHLs

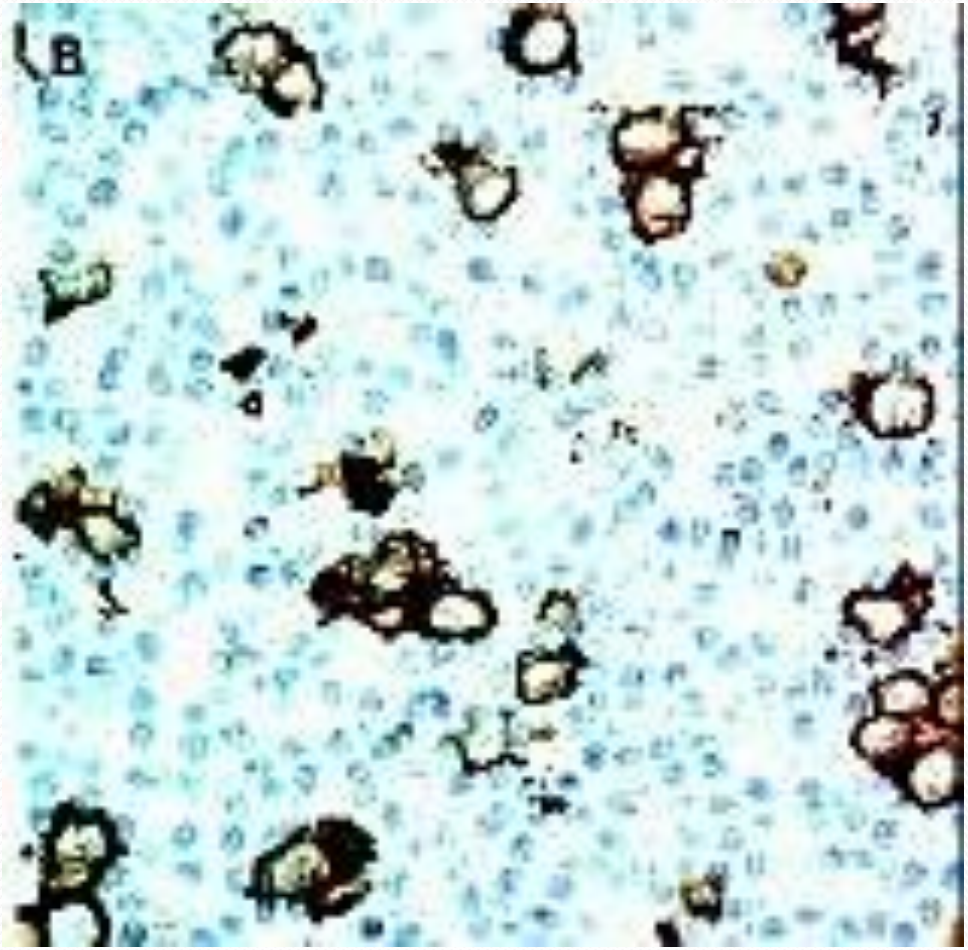
Tools for Tissue Diagnosis

- Proper tissue sampling
- Pathological examination of tissue
  - Morphology by histopathology
  - IPT (Immunophenotyping)
    - IHC (immunohistochemistry)
    - Flow cytometry
  - Cytogenetic and/or molecular genetics (may be needed)

IPT/IHC

- IPT, by no means, replace the role of routine histomorphology but supplement.
- Should be guided by morphology and a battery of markers is almost invariably tested sequentially to reach final diagnosis
- Clinician should provide clinical information but should not instruct which markers to be tested. It should be art of the pathologist to select appropriate markers

- Immunostaining is planned on the basis of morphology
- Immunostaining pattern of morphologically abnormal cell is the subject of consideration, *not* admixed cells or background cells



CD20 immunostainig in T-cell rich B-cell lymphoma

Immunological markers are usually applicable for *classifying* neoplasm, *not* for differentiating neoplastic tissue from normal tissue

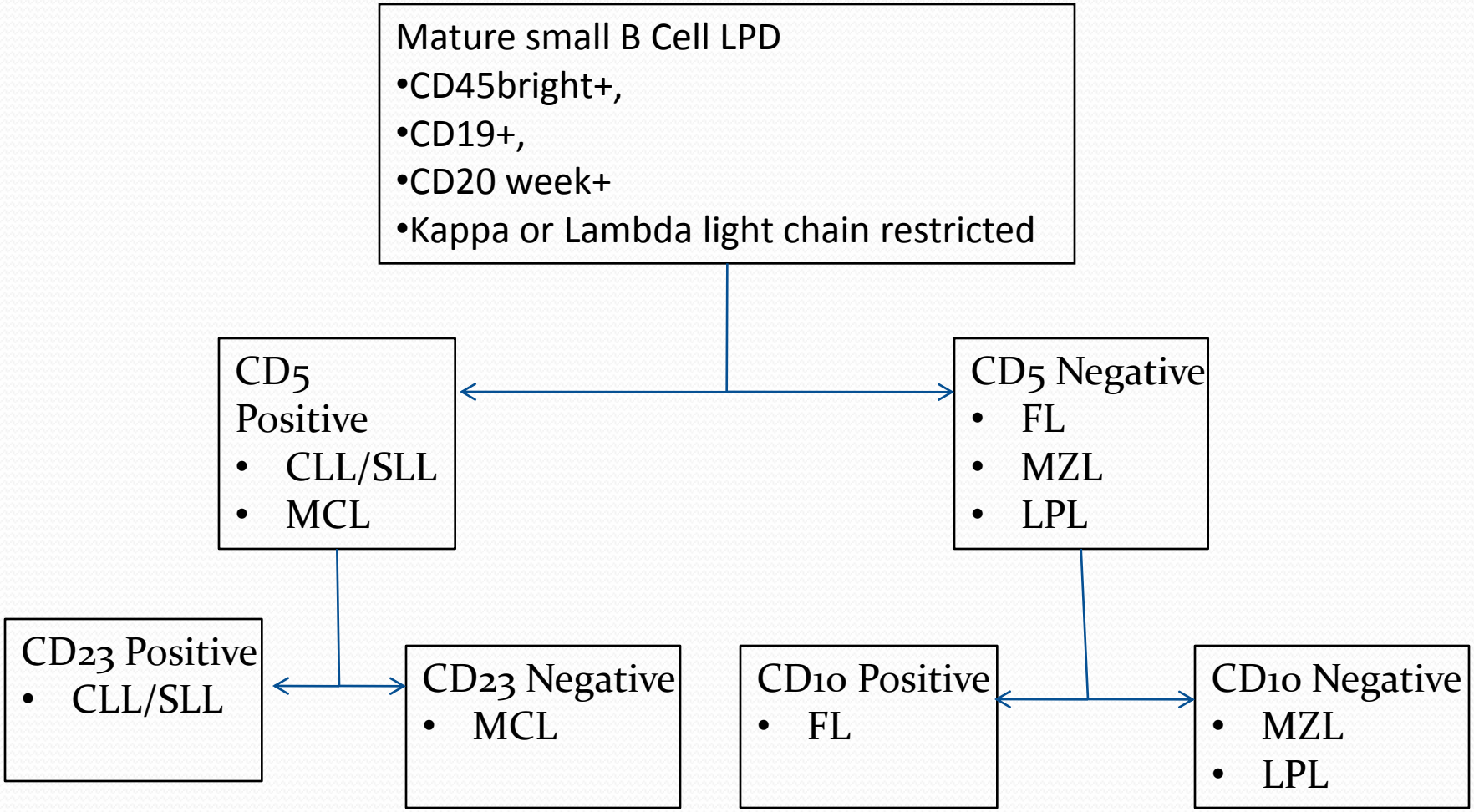
with few exception like bcl2 which help differentiate FL from follicular hyperplasia

IHC is Stepwise Procedure

- 1st step to NHL
  - CD45 is almost invariable in all NHL except in plasmablastic lymphoma
  - Ki67 or MIB1 activity to observe *proliferation index*
- 2nd step
  - CD20: positive in B-cell NHL (exception may be B-lymphoblastic lymphoma)
  - CD3: usually positive in T-cell NHL (exception common especially ALCL)
  - Pax5: All B-cell including truncated B-cell
- Subsequent markers are selected as per previous result

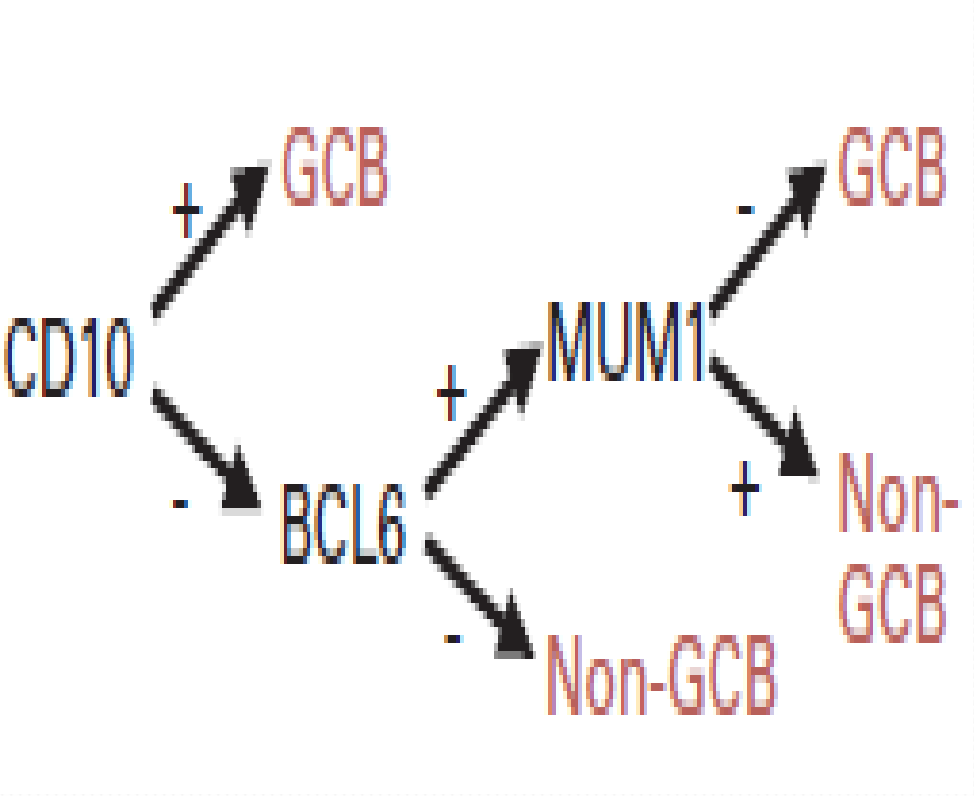
- Significance of proliferation index (Ki67 activity)
  - <35% - indolent lymphomas: small cell lymphomas e.g. FL
  - 45 to 85%- aggressive lymphomas: like DLBCL
  - >95%- very aggressive lymphomas: like lymphoblastic, double hit and Burkit lymphoma
  - Almost 100% is exclusively in Burkit lymphoma

IPT diagnostic flowchart for Small B-Cell Lymphomas



IHC pattern of DLBCL

- CD45, CD19, CD20, CD22, CD79a, monotypic slg positive
- CD3 negative
- Ki67 activity within 45 to 85% usually
- Pax5 positive
- CD30 negative except PMBCL
- tDT negative
- CD10, bcl6 & MUM1 are variable



Hans algorithm for predicting cell of origin in DLBCL

- GCB DLBCL show markedly better outcome in contrast to ABC DLBCL with CHOP therapy

- Proliferation index >95% almost invariably indicate lymphoblastic lymphoma or Burkit lymphoma (BL)
- Presence of immaturity marker tDT is hallmark of lymphoblastic lymphoma
- BL to be confirmed by overexpression of *c-myc* oncogene by FISH
- Double expression lymphoma express both *c-myc* and bcl2/bcl6 oncoprotein in IHC

- Immunophenotypic diagnosis in T-cell and NK-cell lymphomas are much less specific
- There are no universal marker unlike B-cell NHLs
- Variable combinations of markers along with much more emphasis on morphology are required.
- ALK positivity in anaplastic large cell lymphoma (ALCL) confer better prognosis.

Newer treatment regimens of NHL

Evolution of Therapy in DLBCL

- After introduction of **CHOP** (cyclophosphamide, hydroxydaunorubicin, oncovin & prednisolone) in early ’70. No more intensive chemo regimen has been proven to be more effective than CHOP until introduction of **rituximab** in late ’90.
- Addition of **rituximab** with all chemo regimen in the treatment of all B-cell lymphoma/LPD has been shown to be definitely benificial.

Benefit of Addition of Rituximab in Indolent Lymphomas

			Overall survival (%)		
Study	**Regimen**	**Follow-up**	**Control**	**Rituximab**	***p*-value**
M39021[1] (Marcus)	CVP vs R-CVP	4 years	77	83	0.029
GLSG[2,3] (Hiddemann)	CHOP vs R-CHOP	5 years	84	90	0.0493
M39023[4,5] (Herold)	MCP vs R-MCP	4 years	74	86	0.0205
FL2000[6] (Salles)	CHVP/IFN vs R-CHVP/IFN	5 years	79	84	0.025 (high-risk pts)

1. Marcus R, *et al. J Clin Oncol* 2008; 26:4579–4586. 2. Hiddemann W, *et al. Blood* 2005; 106:3725–3732.
3. Buske C, *et al. Blood* 2008; 112:Abstract 2599. 4. Herold M, *et al. J Clin Oncol* 2007; 25:1986–1992.
5. Herold M, *et al. Ann Oncol* 2008; 19(Suppl 4):Abstract 329. 6. Salles G, *et al. Blood* 2008; 112:4824–4831

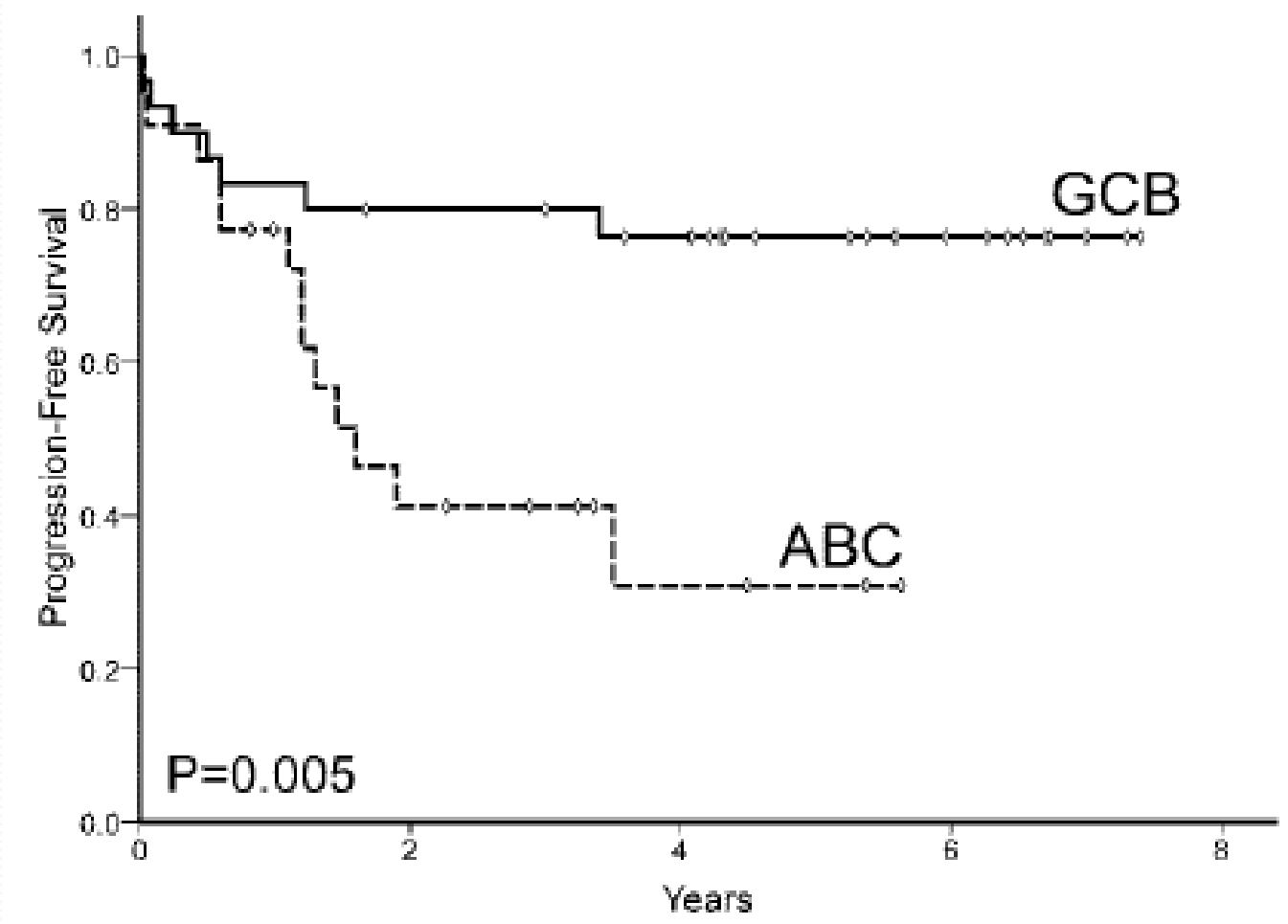
Benefit of Addition of Rituximab in DLBCL

Trials Using Rituximab for Diffuse Large B-Cell Lymphomas in the First-Line Setting

Study	Patient Population	Regimen	Overall Survival	Progression-Free Survival
Coiffier, 2002[45]	(n = 399) Previously untreated Age 60–80 years	R-CHOP vs. CHOP	70% vs. 57%	57% vs. 38%
Pfreundschuh, 2006[49]	(n = 824) Previously untreated Age 18–60 years	R-CHOP-like chemotherapy vs. CHOP-like chemotherapy	93% vs. 84% ($P = 0.0001$)	79% vs. 59% ($P < 0.0001$)
Habermann, 2006[48]	(n = 632) Previously untreated Age > 60 years	R-CHOP vs. CHOP	Not reached	53% vs. 46% ($P = 0.04$)

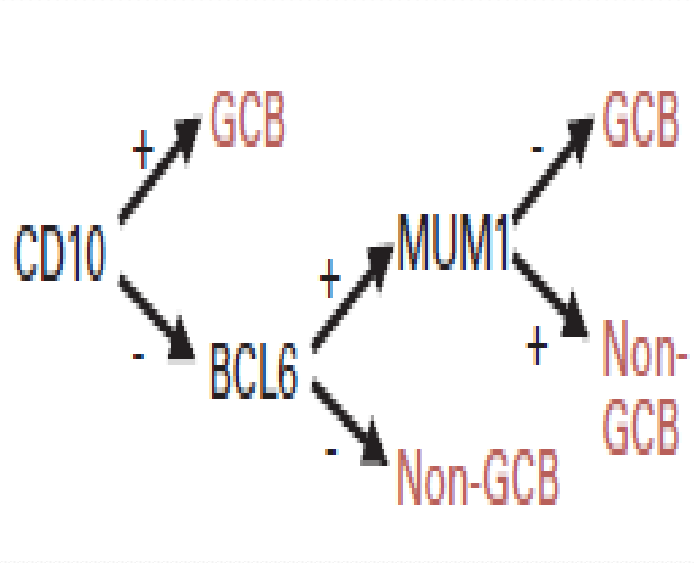
Even after introduction of rituximab, long term survival in DLBCL remained <60% and in some subgroup (ABC type or with high IPI score), results are far more frustrating.

Outcome of GCB vs ABC DLBCL with R-CHOP



However

- Gene expression profile can’t be seen in routine clinical lab
- IPT algorithms, like Hans model can’t differentiate GCB from non-GCB very accurately



So, effort to improve overall outcome, irrespective of risk group, continued.

One such potentially encouraging regimen is DA-EPOCH

DA = Dose adjusted

E = Etoposide

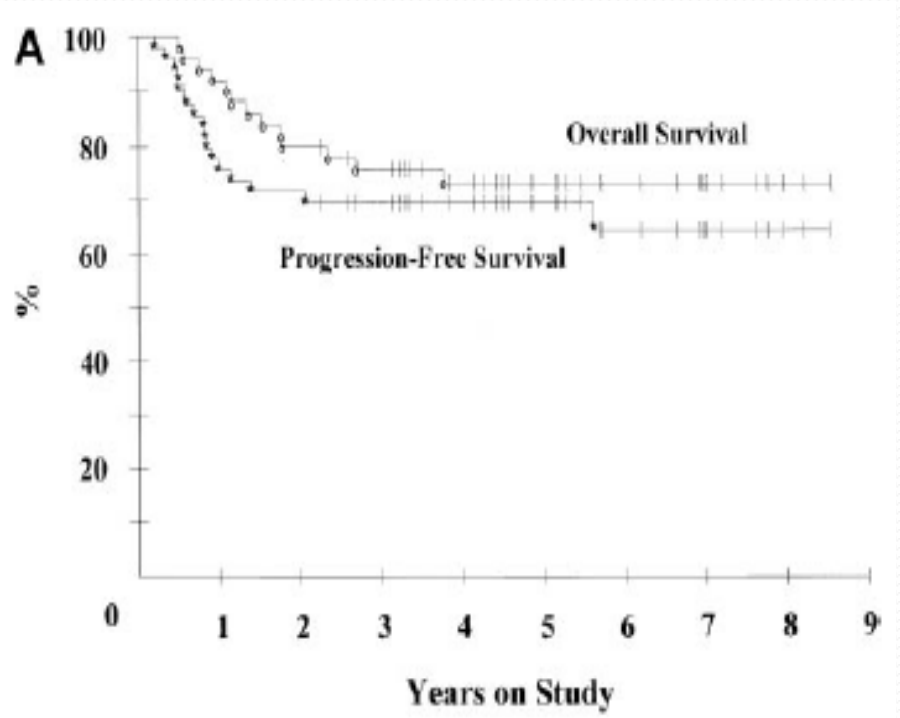
P = Prednisolone

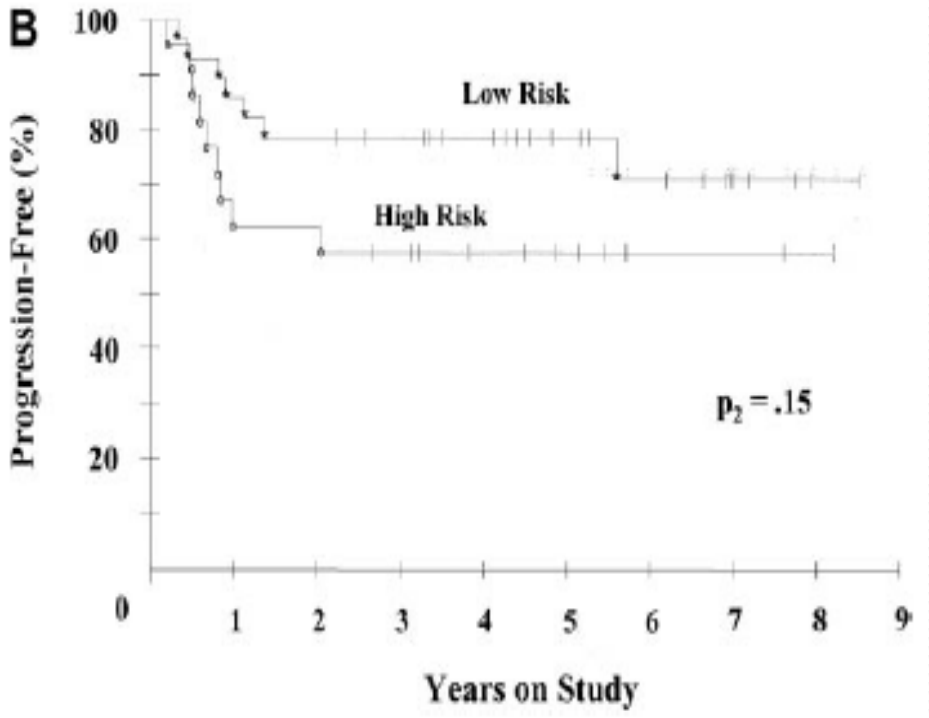
O= Oncovin (vincristine)

C = Cyclophosphamide

H= Hydroxydaunorubicin (or doxorubicin)

Outcome of DA-EPOCH in Brief in Phase II Study (n=49)





Ref: Blood. 2002;99(8):2685-2693

Outcome of DA-EPOCH in Brief in Phase II Study (n=49)

Table 3. Patient characteristics and outcome

Characteristic	No. (%)*	% PFS at 62 mo†	P_2‡	% OS at 62 mo†	P_2‡
Total patients	50 (100)	70		73	
Sex					
Male	25 (50)	71	.92	75	.83
Female	25 (50)	68		71	
Median age, y (range)	46 (20-88)				
Younger than or equal to 60 y	38 (76)	68	.85	78	.14
Older than 60 y	12 (24)	73		58	
Performance status					
ECOG 0 to 1	45 (90)	68	.59	70	.19
ECOG at least 2	5 (10)	80		100	
Disease stage					
I/II	6/7 (26)	85	.47	85	.39
III/IV	9/28 (74)	64		69	
LDH level					
Normal	15 (30)	80	.40	86	.18
Above normal	35 (70)	65		68	
Extranodal sites					
0 to 1	33 (66)	78	.07	81	.09
At least 2	17 (34)	53		58	
IPI score					
Low (0-1)	19 (38)	79	.52	84	§
Low intermediate (2)	9 (18)	78		100	
High intermediate (3)	16 (32)	54		42	
High (4-5)	6 (12)	67		83	

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Those encouraging results of phase II study leads to phase III study to compare DA-EPOCH-R with R-CHOP in DLBCL (CALGB/Alliance 50303)

Results of clinical data presented in 2016 ASH meeting

CALGB/Alliance 50303: Efficacy of R-CHOP versus DA-EPOCH-R in untreated DLBCL

	R-CHOP (n=233)	DA-EPOCH-R (n=232)	*P* value
Best clinical response			**.98**
Overall response rate	89.3%	88.8%	
Complete remission	62.3%	61.1%	
Partial remission	27.0%	27.2%	
Stable disease	2.6%	3.5%	
Progressive disease	1.7%	<1.0%	

R= Rituximab, DA= Dose adjusted
CHOP= Cyclophosphamide, hydroxydaunorubicin, oncovin & prednisolone
EPOCH= Etoposide, prednisolone, oncovin, cyclophosphamide & hydroxydaunorubicin

CALGB/Alliance 50303: Efficacy of R-CHOP versus DA-EPOCH-R in untreated DLBCL (*Continued)*

	R-CHOP (n=233)	DA-EPOCH-R (n=232)	*P* value
Event free survival			**.44**
3 -year	81%	79%	
5 -year	69%	66%	
Overall survival			**.42**
3-year	85%	85%	
5-year	80%	76%	

Ref: Early clinical data presented in ASH annual meeting, 2016.

- Phase III data fail to show significant benefit of DA-EPOCH-R over R-CHOP
- Data regarding molecular, GEP and IPI are yet to be analysed
- A subgroup (adverse) of DLBCL may be benefited from this more intensive approach

Addition of linalidomide (Revlimid) to R-CHOP in attempt to mitigate the bad prognostic effect of ABC origin in DLBCL

Combining linalidomide with R-CHOP: Can it mitigate the negative prognostic index associated with non-GCB phenotype of DLBCL

	R-CHOP (n=87)			R2- CHOP (n=60)		
	GCB	Non GCB	*P*	GCB	Non GCB	*P*
2-year PFS	64%	28%	<.001	59%	60%	.83
2-year OS	78%	46%	<.001	80%	75%	.61

PFS= Progression free survival, OS= Overall survival, R= Rituximab,
R2= Revlimid (linalidomide) and rituximab
CHOP= Cyclophosphamide, hydroxydaunorubicin, oncovin & prednisolone

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Combining linalidomide with R-CHOP can mitigate the negative prognostic index associated with non-GCB phenotype of DLBCL

Relapsed/Refractory DLBCL

- Salvage therapy consolidated with myeloablative therapy followed by ASCT only curative option
- The requirement of such approach can be minimized only with improvement of 1st line treatment

Indolent B-cell Lymphomas (e.g. Follicular Lymphoma)

- Indolent lymphomas are incurable, but show much more prolonged survival
- May not require treatment for prolonged period
- May undergo multiple remission and relapse and finally transformation
- Those properties of indolent lymphoma provide ideal soil for development of newer targeted therapy in lymphoma which may be applicable in aggressive lymphomas as well subsequently

Some such targeted therapeutic agents already approved are

- B-cell receptor pathway inhibitors
  - Ibrutinib
  - Idelalisib
- BCL2 inhibitor
  - Venetoclax
- Type II anti CD20
  - Obinutuzumab
  - Ofatumumab

Newer approach of treatment of PTCL

- Scarcity of targeted therapy like rituximab in PTCL
- With CHOP therapy all type of PTCL show markedly worse 5 year FFS (18 to 36 %) except ALK positive ALCL
- Several intensified regimen failed to add any benefit but added toxicities
- Addition of **etoposide** for 3 days to CHOP (CHOEP) has showed significant benefit (3 year event free survival 75.4% vs 51% with CHOP) in <60 year old subgroup only

Only targeted therapeutic agent for T-cell lymphoma is anti-CD30 *brentuximab vidotin,* applicable in CD30 positive lymphomas, e.g. ALCL

Useful in classical HD as well

Newer approach of treatment of PTCL *Cont*

- So, upfront ASCT is recommended as standered of care in all PTCL except ALK positive ALCL

Take Home Messages

- Immunophenotyping is the most important and virtually essential tool for categorizing lymphomas correctly.
- However routine morphology still remain indispensable
- Benefit of intensification of chemotherapy may be offset by added toxicities.
- Properly identifying the subgroup with poor prognosis and tailoring therapy accordingly is prudent approach.
- Developing newer targeted therapies are future directives with promising outcome and minimized toxicities.

Thanks for Patience Hearing