

## Proceedings

# Precision Pathology of Lymphoid Neoplasms

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**Abstract:** This section is dedicated to the precision pathology of lymphoid neoplasms. Dr. Mariusz Wasik, in his keynote presentation *NPM::ALK*: The prime example of potent oncogene and therapeutic target, discussed the critical role ALK1 plays in the pathogenesis of anaplastic large T-cell lymphoma (ALCL), why it serves as an optimal therapeutic target, and how to overcome the drug resistance. Dr. Deniz Peker Barclift from the pathological diagnosis to detection of measurable residual disease presented the molecular applications in the diagnosis and precision therapy of chronic lymphocytic leukemia (CLL). Dr. Xianfeng F. Zhao finally reviewed the recent molecular advances in diffuse large B-cell lymphoma (DLBCL) and shared his perspective on the precision pathology of DLBCL. These presentations together illustrate an exemplary landscape of precision pathology on both indolent and aggressive malignant lymphomas.

**Keywords:** ALCL, ALK1, CLL, DLBCL, NPM, Precision pathology

## ***NPM::ALK*: The Prime Example of Potent Oncogene and Therapeutic Target**

*ALK1* gene itself and its constitutively active oncogenic gene-fusion form *NPM::ALK*, were first identified in anaplastic large cell lymphoma (ALCL) of CD4+ T-cell origin [1]. As a member of tyrosine kinase family, ALK1 is a cell-membrane receptor physiologically expressed only in fetal neural cells, and it is important for differentiation and survival of these cells [2]. ALK1 is related to LTK and ROS, and remotely to insulin receptor. Follow-up studies

identified a number of different *ALK1* gene translocations with diverse partners in the whole spectrum of malignancies and in neuroblastoma, hence a tumor closely resembling fetal neural cells, full length of ALK1 can also acquire activating point mutations in the kinase domain (Table 1). Accordingly, in addition to ALCL, aberrant *ALK1* expression has been detected in multiple malignancies which include B-cell lymphomas, inflammatory myofibroblastic tumor (IMFT), non-small cell lung carcinoma (NSCLC), renal medullary carcinoma (sickle cell trait-associated), anaplastic thyroid carcinoma, and neuroblastoma (sporadic and familial).

In regard to the underlying mechanism of *NPM-ALK* oncogenicity, *ALK1* becomes aberrantly and constitutively expressed due to persistent activation of the *NPM* promoter in the host CD4+ T cells and the *NPM-ALK* protein is constitutively

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**Table 1:** ALK Inhibitors, Indications, and Active ALCL Clinical Trials

Drug	Company	Generation	FDA approval	Active ALCL clinical trials	Note
<b>Crizotinib/</b> Xalkori	Pfizer	1st	Locally advanced and metastatic NSCLC (US)	NCT00939770	Phase I/II: pediatric ALCL, CNS tumors (monotherapy)
				NCT02487316	Phase IV: adult ALCL (+/- chemotherapy)
				NCT01606878	Phase I: pediatric ALCL (combination with chemotherapy)
				NCT02584634	Phase I: adult NSCLC (combination avelumab with crizotinib or lorlatinib)
<b>Ceritinib/</b> LDK378	INovartis	2nd	Metastatic crizotinib-resistant NSCLC (US)		
<b>Alectinib/</b> CH5424802/ AF802	Roche/ Chugai	2nd	Crizotinib-resistant NSCLC (US); ALK+ NSCLC (Japan)	NCT01588028	Phase I: ALK+ NSCLC
<b>ASP3026</b>	Astellas	2nd		NCT01284192	Phase I: adult refractory solid tumor or B cell lymphoma
<b>Brigatinib/</b> AP26113	Ariad	2nd		NCT01449461	Phase I/II: adult NSCLC, ALCL, DLBCL, IMFT
				NCT02094573	Phase II: adult NSCLC
<b>Lorlatinib/</b> PF- 06463922	Pfizer	3rd		NCT01970865	Phase I/II: adult ALK+ and ROS1+ NSCLC
				NCT02584634	Phase I: adult NSCLC, avelumab (with crizotinib or lorlatinib)
				NCT02569554	Phase I: adult, healthy, PPI and food effect

Abbreviations: ALK, anaplastic lymphoma kinase; ALCL, anaplastic large-cell lymphoma; CNS, central nervous system; DLBCL, diffuse large-cell lymphoma; IMFT, inflammatory myofibroblastic tumor; NSCLC, non-small cell lung cancer; PPI, proton pump inhibitor; US, United States.

activated due to the NPM domain-mediated NPM-ALK oligomerization, resulting in the ALK kinase domain-triggered autophosphorylation [3]. NPM-ALK is highly oncogenic, both *in vitro* and *in vivo* in mouse models [3]. Experiments showed that induced expression of *ALK1* results in malignant transformation of normal human CD4+ T lymphocytes; the cells became immortalized and larger in size [4]. These *NPM-ALK* transduced T cells are completely NPM-ALK-dependent, because their treatment with either ALK inhibitor or siRNA affects their size and

inhibits their growth. In the xenografted animal models, the *NPM-ALK* transfected CD4+ T cells recapitulate the morphology and immunophenotype of human ALCL [4]. ALK+ ALCL patient studies have shown that of the 11 adult patients with advanced/therapy-resistant ALCL treated with a single agent, first-generation ALK inhibitor crizotinib, 7 achieved complete remission (CR) with 3 undergoing follow-up allotransplant consolidation [5,6]. Discontinuation of ALK inhibitor therapy in the responders resulted in disease recurrence [7]. In

the pediatric patients treated with ALK inhibitors, 7 out of 9 patients achieved CR; some with just one cycle of treatment, with 3 undergoing autologous bone marrow transplant. In the follow up study of 26 pediatric/young adult patients, >80% patients achieved CR [8,9]. Due to the high efficacy, FDA approved crizotinib for ALK+ ALCL therapy in January 2021. Unfortunately, drug resistance frequently develops, mainly through *ALK* mutations [9]. New generations of ALK1 inhibitors have been produced and sequential treatment may be able to overcome the newly emerging drug-resistant mutations (Table 2) [10]. However, the drug resistance remains a challenge and multiagent novel combination therapy may well prove a strategy necessary to prevent the occurrence of drug resistance.

NPM-ALK activates several downstream signal transduction pathways [11], which include PI3K-

AKT-FOXO3A/p27, mTOR, MEK-ERK, and JAK-STAT. NPM-ALK hijacks the preexisting signaling pathways, physiologically activated by IL2-type cytokines to promote the development and growth of ALCL [11]. Interestingly, ALK-negative ALCL uses a similar mechanism by activating *ROS1*, *TYK2*, and mutated *JAK1* to eventually activate STAT3 in the transformation of normal T-cells [12,13]. Recent study showed that *JAK2* translocations also play a role in activating the downstream cytokine pathways in the ALK-negative ALCL [14]. Through inducing epigenetic silencing of *SHIP-1*, *STAT5A*, and *IL-2R $\gamma$* , *NPM-ALK* protects its own constitutive expression [15-17], while it also promotes expression of DNMT1, the DNA-modifying enzyme needed to silence these genes [18]. By inhibiting IL2-type and T-cell receptor (TCR) signaling, NPM-ALK mediates the reprogramming of CD4+ T cells from TCR

**Table 2:** Resistant and Sensitive Mutations of ALK-TKIs

ALK	Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib	Gilteritinib
Sensitive mutations	L1198F	G1269A/S	C1156Y/T	C1156Y/T	C1156Y/T	I1171N
	C1196Y+L1198F	I1171T/N	F1174C/L/V	E1210K	E1210K	I1171N+F1174I
	C1156Y+L1198F	L1196M	G1269A/S	F1174C/L/V	F1174C/L/V	I1171N+L1198H
		S1206C/Y	I1151Tins	F1245A	G1269A/S	
		I1171S+G1269A	L1196M	G1202R(-)	G1202R	
			L1256F	G1269A/S	I1151Tins	
			L1152P/R	I1151tins	L1152P/R	
			S1206C/Y	L1152P/R	L1196M	
			I1171N+L1256F	L1196M	S1206C/Y	
				I1171S+G1269A		
Resistance mutations	C1156Y/T	C1156Y/T	G1202R	D1203N	C1156F/Y	
	E1210K	F1174C/L/V	I1171T/N/S	G1202R(-)	L1198F	
	F1245V	I1151Tins	V1180L	I1171T/N/S	L1256F	
	G1269A/S	G1202R	L1196M+G1202R	V1180L	G1202R+L1196M	
	G1202R	V1180L		L1196M+G1202R	C1196Y+L1198F	
	I1151Tins	I1171N+F1174I			G1202R+T1151M	
	I1171N/S/T	I1171N+L1198H			G1202R+F1174C	
	L1196M	L1196M+G1202R			I1171N+F1174I	
	L1152P/R				I1171N+L1198H	
	S1206Y/C				L1196M/D1203N	
	V1180L				F1174L/G1202R	
	E1210K+D1203N				C1156Y/G1269A	
	L1196M+G1202R					

and IL-2-type signaling dependence to NPM-ALK dependence. ALK+ ALCL cells are protected from immune response by both secreting tolerogenic cytokines (IL-10 & TGF $\beta$ ) and expressing immunosuppressive cell-membrane protein CD274 (PD-L1) [19,20]. Of note, a chemotherapy- and ALK inhibitor-resistant ALK+ ALCL patient responded to anti-PD1 antibody and achieved complete remission [21], identifying potential novel therapy for ALK+ALCL and, perhaps, other ALK-driven malignancies. Study also showed that NPM-ALK induces expression of ICOS gene: “parasitic” utilization of stimulatory signals generated by immune cells [22] and expression of *HIF1 $\alpha$*  mRNA to protect malignant cells from effects of hypoxia [23]. It also induces expression of genes encoding stem-cell type transcription factors: TWIST1, SOX2, and SALL4 to confer the CD4+ T-cell features of immaturity [24-27], as well as the loss of T-cell identity [28]. NPM-ALK induces protection of lymphoma cells from apoptotic death via STAT3-HES1-TXNIP network [29].

In summary, NPM-ALK transforms CD4+ T cells by 1) constantly activating IL-2-type signaling pathways; 2) regulating key cell functions that include cell growth and hypoxia tolerance via HIF-1 $\alpha$ ; 3) modulating immune system (IL-10, TGF $\beta$ , CD274/PD-L1, and ICOS) to suppress anti-tumor response; 4) affecting epigenetics via STAT3-promoted expression of DNMT1 by inducing transcription of *DNMT1* gene and by suppressing expression of its translational inhibitor *miR-21*; 5) inducing via STAT3 epigenetic silencing of tumor suppressor genes *SHP-1*, *STAT5A*, and *IL-2R $\gamma$*  to protect its own expression; 6) inducing via STAT3 expression of *HES1* to inhibit the expression of pro-apoptotic tumor suppressor *TXNIP*, and enhancing cell metabolism by activating mTORC1. Therefore, NPM-ALK is an omnipotent oncogene and serves as an optimal target for precision cancer therapy.

## Molecular Applications in Chronic Lymphocytic Leukemia (CLL): From Diagnosis to Detection of Measurable Residual Disease

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is an indolent neoplasm of mature B-cells. CLL is the most frequent leukemia in Western countries with 5 cases per 100,000 people. It is more common in Caucasians and has a slight male predilection. Familial predisposition exists with 2- to 8-fold increased risk in first degree relatives. CLL often has a stable course with risk of an aggressive disease and transformation to diffuse large B-cell lymphoma (Richter transformation). Diagnosis of CLL requires a comprehensive full body work up including blood and bone marrow examination using histomorphology, flow cytometry, molecular testing and body imaging to determine the disease extension. For diagnosis of CLL, monoclonal B-cells should be equal or more than  $5 \times 10^9$ /L in blood or, if less than  $5 \times 10^9$ /L, should be accompanied by organomegaly and/or extramedullary disease. Blood and bone marrow show lymphocytosis with small, mature lymphocytes displaying condensed chromatin with “soccer ball” appearance and scant cytoplasm. Admixed few prolymphocytes can be observed and should not be in increased numbers. Flow cytometric analysis displays a typical immunophenotype with expression of CD5, CD23 and CD200 in B-cells in most cases. ZAP70 and CD38 expression can be determined by flow cytometry and are used as prognostic indicators in CLL as they reflect *IGHV* mutation status. LEF1 immunohistochemical stain has been used to determine CLL/SLL origin, and cyclin D1 and SOX11 should not be expressed. Molecular testing in CLL is important, particularly at the time of first diagnosis and should include cytogenetics, fluorescence in situ hybridization (FISH) and, preferably, mutation analysis for *TP53* gene (please see Table 3 for frequent cytogenetic abnormalities in CLL and

prognostic implications). Results of these testing are used in CLL risk stratification in the clinical setting.

*IGHV* mutation status is a useful prognostic parameter and potentially influences the type of therapy, i.e. immunotherapy *vs.* novel agent-based therapy. However, since the mutational status considered to be stable over the course of the disease, serial testing is generally not required. Next generation sequencing (NGS) has been used in CLL; however, it is still not a widely used diagnostic test. In addition to *TP53* gene, *SF3B1*, *ATM*, *NOTCH1*, and *BIRC3* genes were shown to be important prognostic indicators in CLL [30], particularly in determining effective treatment. Approximately 8% of CLL patients have *TP53* alterations in the form of *TP53* deletion, *TP53* mutation, or harboring both, and are associated with inferior clinical outcome. It is crucial to determine the status of this gene as chemoimmunotherapy is not recommended in these patients and other regimens should be considered as recommended by NCCN Guidelines 2022 (Table 4). Recent studies have shown that *SF3B1* and *BIRC3* mutations, like *TP53*, are associated with inferior clinical outcome and fludarabine refractory disease [31]. Emerging data from a clinical trial indicate that targeted therapeutic agents may be considered in these patients, such as Bruton tyrosine kinase (BTK) inhibitors and BCL2 inhibitor venetoclax [32].

Minimal measurable or residual disease (MRD) is an emerging parameter to determine treatment response in CLL patients and current data [33] suggest that partial clinical response with undetectable MRD might have superior overall prognosis when compared to complete clinical response with MRD (Figure 1). Therefore, more effective regimens to achieve complete remission without detectable MRD have been investigated. Results of a recent clinical trial showed promising results with combination of acalabrutinib, venetoclax, and obinutuzumab as frontline treatment to reach complete remission without detectable MRD in CLL patients regardless of *TP53* and *IGHV* mutation status [34]. Currently, flow cytometry is the most widely used technique

to measure MRD with alternative testing including allele-specific oligonucleotide PCR and NGS.

## Precision Pathology of Diffuse Large B-Cell Lymphoma (DLBCL)

As the most common type of non-Hodgkin lymphoma in the United States, DLBCL is a heterogeneous group of lymphoid malignancies [35], and the treatment remains largely rituximab + (cyclophosphamide, hydroxydaunorubicin, vincristine sulfate, and prednisone) (RCHOP) with some variations. The current regimen and dosage are based on numerous clinical trials; unfortunately, only 60-70% respond to the regimen and 40-50% of patients relapse [36]. Therefore, novel therapies are badly needed for DLBCL, which include targeted therapy.

DLBCL has been classified through years with its vividly descriptive name. Based on gene profiling data [37,38], WHO Classification currently divides DLBCL into three major subtypes: 1) activated B-cell like (ABC) subtype (with a 5-year survival rate of 35%); 2) germinal center B-cell like (GCB) subtype (with a 60% 5-year overall survival rate); and 3) unclassified subtype (with a 5-year survival rate of 39%). GCB subtype DLBCL is associated with perturbations in the PI3K/AKT and JAK/STAT signaling pathways and a higher frequency of *BCL2*, *EZH2* and *MYC* mutations, whereas ABC subtype DLBCL is characterized by activation of the BCR-mediated NF- $\kappa$ B and PI3K/AKT pathways, and a higher frequency of *BCL6* rearrangements, *BCL2* amplifications, and recurrent mutations of *CD79B*, *MYD88*, and *PRDM1* [39,40]. Using shared genetic abnormalities, DLBCL has recently been classified into four prominent genetic subtypes [41]: 1) MCD subtype (based on the co-occurrence of *MYD88L265P* and *CD79B* mutations); 2) BN2 subtype (based on *BCL6* fusions and *NOTCH2* mutations); 3) N1 subtype (based on *NOTCH1* mutations); and 4) EZB subtype (based on *EZH2* mutations and *BCL2* translocations).

The most significant advance in DLBCL targeted

**Table 3:** Prognostic Value of Chromosomal Aberrations in CLL

Aberration	Frequency	Prognostic implication
Del13q14	>50%	Low risk
Trisomy 12	16%	Intermediate risk (w or w/o del13q14) Increased risk with <i>NOTCH1</i> mutations Present in 1/3 of Richter transformation cases
Del11q23	5-20%	Bulky, progressive and refractory disease Commonly encompasses <i>ATM</i> gene
Del17p13	4-8% at diagnosis 10-12% at the 1st therapy 30-40% relapse following FCR	Anomalies in <i>TP53</i> gene Refractory disease Resistance to fludarabine-based chemoimmunotherapy

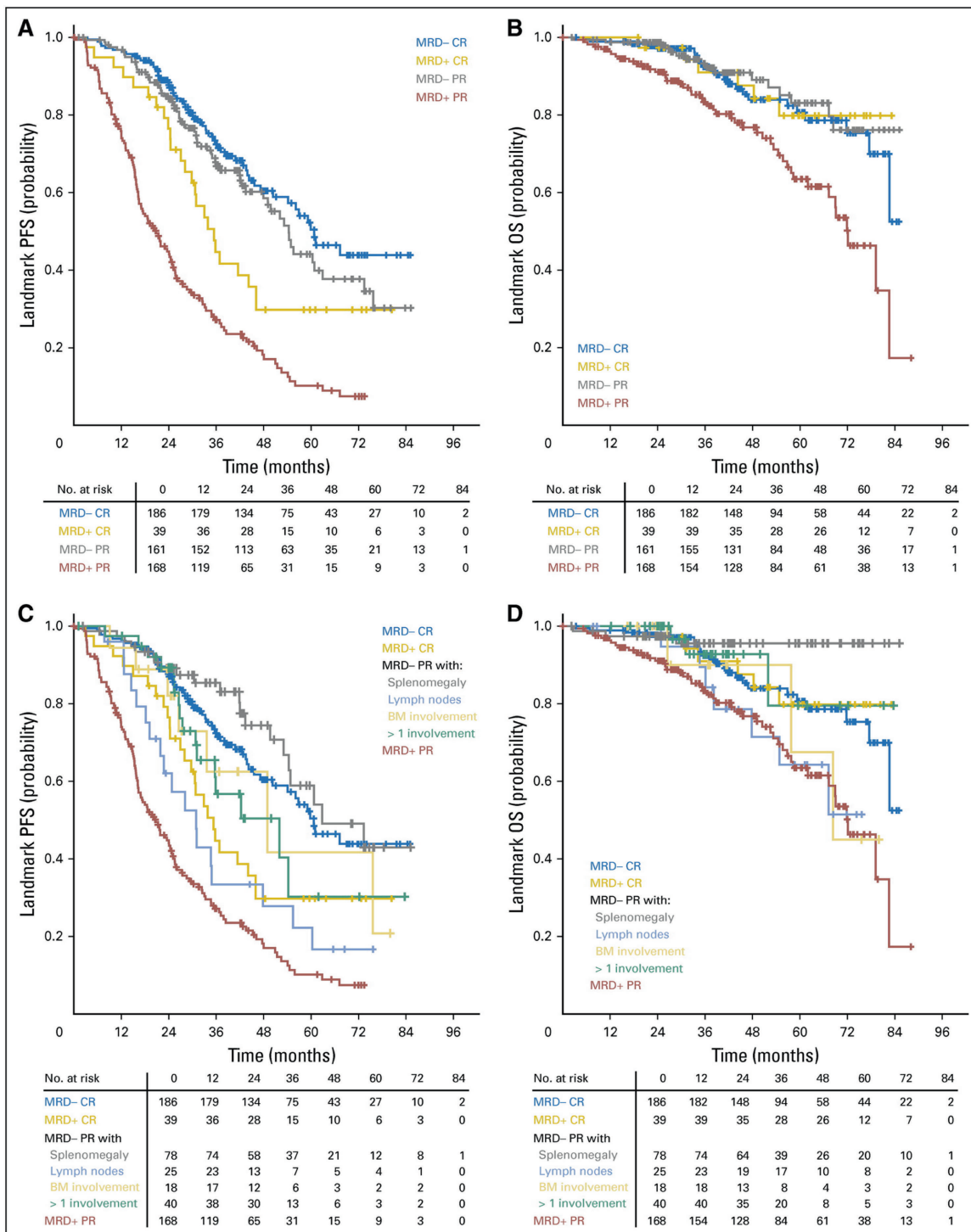
therapy is the ibrutinib effect on DLBCL [42]. Ibrutinib, a BTK inhibitor, in combination with RCHOP gained a 100% response rate in a subgroup of young patients and the response is highly dependent on the mutations fostering the B-cell receptor (BCR) signaling and BTK dependence [43]. It is not known if ibrutinib by itself would gain a similar response without RCHOP. Another study by the same team found that the sole agent ibrutinib inhibitory effect on ABC subtype DLBCL indeed depended on the activated BCR signaling rather than the *MYD88* mutation. However, when the mutated *MYD88* became

independent of BCR activation, ibrutinib lost its inhibitory effect on the ABC subtype DLBCL, even with NF- $\kappa$ B pathway activation [44].

With increasing popularity of precision medicine, many studies are being performed on targeted agents for the relapsed and refractory DLBCL [45]. However, these studies have some issues. For example, since most of the studies are for either relapsed or refractory diseases, it is hard to evaluate the effects on primary DLBCL. Considering all the medical conditions, the studies did not include pharmacomics which is critical for precision medicine.

**Table 4:** NCCN Guidelines (2022) Recommended Treatment Regimens for CLL/SLL with Del(17p)/*TP53* Mutation

FIRST-LINE THERAPY	
Preferred regimens	Other recommended regimens
Acalabrutinib $\pm$ obinutuzumab	Alemtuzumab $\pm$ rituximab
Ibrutinib	HDMP + rituximab
Venetoclax + rituximab	Obinutuzumab
Ibrutinib	HDMP + rituximab
Venetoclax	
Ibrutinib	HDMP + rituximab
Zanubrutinib	
SECOND-LINE AND SUBSEQUENT THERAPY	
Preferred regimens	Other recommended regimens
Acalabrutinib (Category 1)	Alemtuzumab $\pm$ rituximab
Ibrutinib (Category 1)	Duvelisib
Venetoclax + rituximab (category 1)	HDMP + rituximab
Venetoclax	Idelalisib $\pm$ rituximab
Zanubrutinib	Lenolidomide $\pm$ rituximab
	Ofatumumab



**Figure 1: PFS (A and C) and OS (B and D) at end of treatment by peripheral blood MRD and additional response status.** BM, bone marrow; CR, complete response; MRD-, minimal residual disease negative; MRD+, minimal residual disease positive; OS, overall survival; PFS, progression-free survival; PR, partial response (Reprinted from [33] under the CC BY4.0: <https://creativecommons.org/licenses/by/4.0/>).

Population-based dosages led to incorrect efficacy and toxicity in individualized patients. Most of the studies are combined with non-targeting agents [42,43,45] and the sole effect of the targeting agents are not well evaluated. Current clinical trials are still population-based, which could misinterpret the clinical outcomes in individual patients. Due to these issues, the current classification has been questioned by clinicians [46,47], and it seems that the traditional classification no longer meets the requirement of precision medicine.

Previous study showed that treatment of lymphoma should be individualized [48]. In an investigation of DLBCL transformation, amplified *RPS6KB1* and *CDC2* genes were identified in the transformed large B-cell lymphoma by comparative genomic hybridization (CGH). Further study showed that the activated *RPS6KB1* product, p70S6K, and *CDC2* serine/threonine kinases were abundant in DLBCL specimens [48], and thus the activated (phosphorylated) p70S6K and *CDC2* could be therapeutic targets for DLBCL. When four DLBCL cell lines were treated with rapamycin and/or UCN-01 for 5 days, viable lymphoma cells were markedly decreased. Except for Toledo cells, the other 3 cell lines showed brief inhibition and subsequent recovery of cell proliferation with single agents (Figure 2). When combined, rapamycin and UCN-01 synergistically inhibited proliferation of both Pfeiffer and Toledo DLBCLs. Analysis of cell cycle progression of the two DLBCL cell lines revealed that UCN-01 effect dominated in Pfeiffer cells, whereas G1 arrest dictated Toledo cells with predominant rapamycin effect (Table 5). Apoptosis played a major role in Pfeiffer cells but a minor role in Toledo cells. Although DLBCL cell proliferation were inhibited in both Pfeiffer and Toledo cells, they employed different mechanisms; in Pfeiffer cells, the dominant effect of UCN-01 caused apoptosis whereas in Toledo cells, rapamycin led to G1 arrest. Furthermore, rapamycin also inhibited the phosphorylation of p70S6K in Toledo cells, but not in Pfeiffer cells.

In addition to the PI3K/AKT/mTOR/p70S6K

pathway, *ERK1/2* and *CHK2* genes were also overexpressed in DLBCL cells [49]. Either single agents or in combination, *ERK1/2* and *CHK2* inhibitors led the DLBCL cells to apoptosis. When the inhibitors combined, the apoptosis was more prominent in both the DLBCL cell lines and the xenografted tumors of the SCID mouse model. Crosstalk between the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways has been implicated in cancer pathogenesis and targeted therapies [50,51]. Therefore, multi-agent combination therapy is likely an effective strategy to overcome drug resistance [52].

For precision diagnosis of DLBCL, excisional biopsy is preferred whenever possible since more tumor cells will be available for various studies. The specimen could be divided into at least three parts for: 1) morphology and immunophenotyping for pathological diagnosis; 2) NGS mutational analyses of the BCR, NF- $\kappa$ B, JAK/STAT, BCL2, BCL6, MYC, PI3K/AKT/mTOR/p70S6K, and RAS/RAF/MEK/ERK pathways and other critical components; 3) lymphoma cell culture for selecting therapeutic agents and evaluating the efficacy. Once the drugs are selected, draw patient's blood for pharmacomics to determine the metabolizing rate, xenobiotic effects, drug-drug interactions, and adverse effects of the therapeutic drugs.

Therefore, the future pathology report should include: a) pathology diagnosis based on morphology and immunophenotype; b) NGS results for BCR, NF- $\kappa$ B, JAK/STAT, PI3K/AKT/mTOR/p70S6K, and RAS/RAF/MEK/ERK pathways and other critical components; c) activated pathway kinase profiles; d) efficacy of selected drug(s) on lymphoma cells; e) pharmacomics of patient; f) drug toxicity and other adverse effects on the patient.

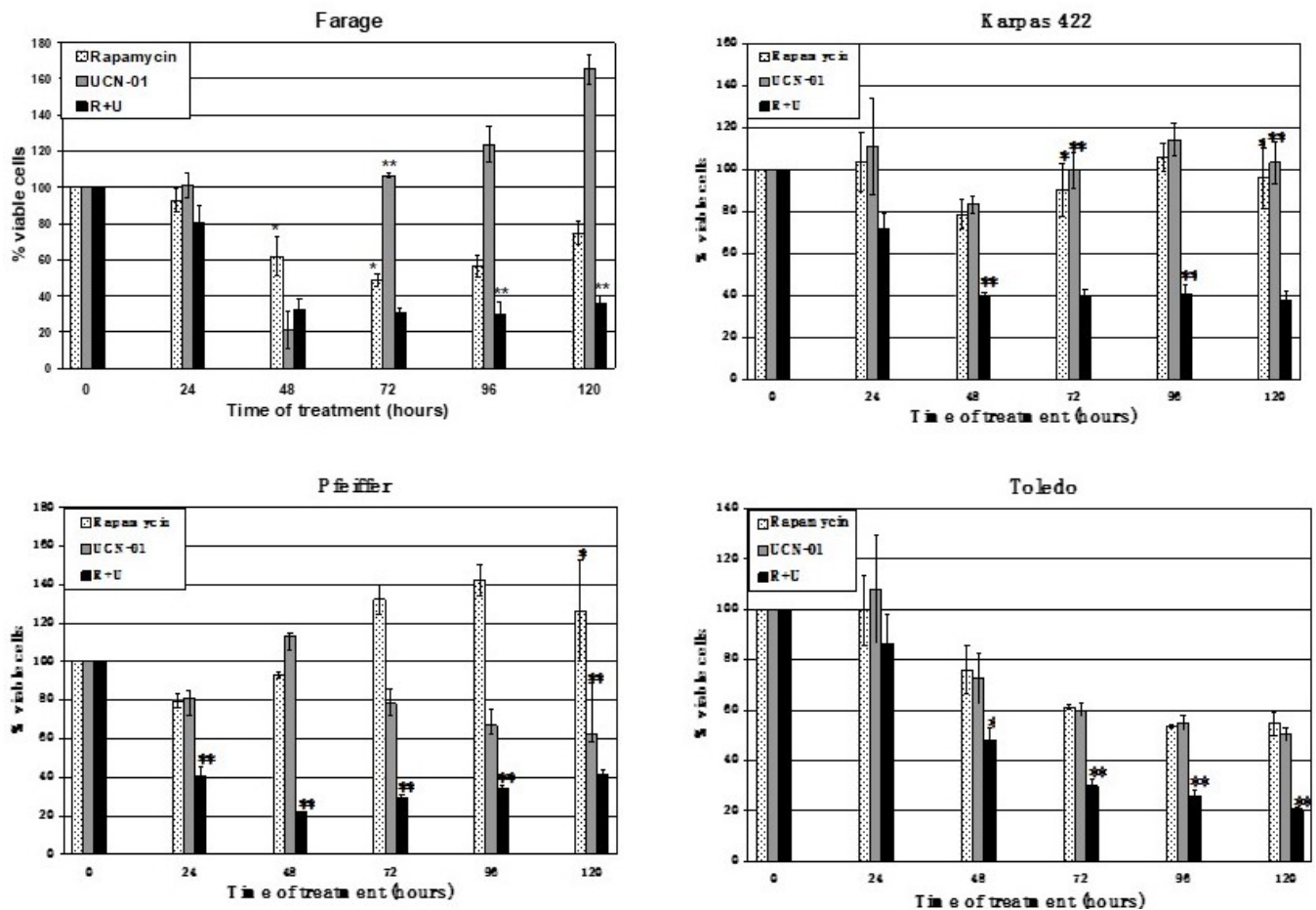
Future clinical trials should be individualized which include screening the drugs that inhibit proliferation of the DLBCL cells with high efficacy and screening the patients with pharmacomics to identify the subjects who more likely will respond with minimal toxicity in the body. The other patients, even though with the same diagnosis, should not be



**Table 5:** Effects of Rapamycin and UCN-01 on Pfeiffer and Toledo DLBCL Cells

Cells	Effect	Rapamycin	UCN-01	Combined
Pfeiffer	Apoptosis	-	++	+++
	G1 arrest	+	-	-
	CDC2 suppression	+	+	++
	p70S6K dephosphorylation	-	-	-
Toledo	Apoptosis	-	-	+
	G1 arrest	++	+	++
	CDC2 suppression	++	++	++
	p70S6K dephosphorylation	++	-	+++

UCN-01, 7-hydroxystaurosporine.



**Figure 2:** Different responses to rapamycin and UCN-01 treatment by DLBCL cells. Except for Toledo cells, the other 3 DLBCL (Farage, Karpas 422, and Pfeiffer) cell lines showed brief inhibition and then recovery of cell proliferation when being treated with single agents. When combined, rapamycin and UCN-01 consistently inhibited the proliferation of all the DLBCL cell lines (please see [48] for further detail).

included in the clinical trial.

In summary, precision pathology of DLBCL remains a challenge for pathologists. For the best clinical outcome of patients, we should use all the molecular data including pathway and cell cycle kinase activities to select the most efficacious drugs, and pharmacomics results to identify patients who benefit most from the selected therapeutic drugs. The “trial and error” clinical practice should be ended.

## Acknowledgements

The authors claim no conflict of interest with the presentations.

**Received:** March 31, 2022 **Accepted:** April 15, 2022

**Published:** May 10, 2022

## References

- Morris SW, Kirstein MN, Valentine MB, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science*. 1994;263:1281-1284.
- Yao S, Cheng M, Zhang Q, Wasik M, Kelsh R, Winkler C. Anaplastic lymphoma kinase is required for neurogenesis in the developing central nervous system of zebrafish. *PloS one*. 2013;8:e63757.
- Werner MT, Zhao C, Zhang Q, Wasik MA. Nucleophosmin-anaplastic lymphoma kinase: the ultimate oncogene and therapeutic target. *Blood*. 2017;129:823-831.
- Zhang Q, Wei F, Wang HY, et al. The potent oncogene NPM-ALK mediates malignant transformation of normal human CD4(+) T lymphocytes. *Am J Pathol*. 2013;183: 1971-1980.
- Gambacorti-Passerini C, Messa C, Pogliani EM. Crizotinib in anaplastic large-cell lymphoma. *N Engl J Med*. 2011;364:775-776.
- Gambacorti-Passerini C, Farina F, Stasia A, et al. Crizotinib in advanced, chemoresistant anaplastic lymphoma kinase-positive lymphoma patients. *J Natl Cancer Inst*. 2014;106:djt378.
- Gambacorti-Passerini C, Mussolin L, Brugieres L. Abrupt relapse of ALK-positive lymphoma after discontinuation of Crizotinib. *N Engl J Med*. 2016;374:95-96.
- Mossé YP, Lim MS, Voss SD, et al. Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study. *Lancet Oncol*. 2013;14:472-480.
- Mossé YP, Voss SD, Lim MS, et al. Targeting ALK with Crizotinib in pediatric anaplastic large cell lymphoma and inflammatory myofibroblastic tumor: A Children's Oncology Group Study. *J Clin Oncol*. 2017;35:3215-3221.
- Wang Y, He J, Xu M, et al. Holistic view of ALK TKI resistance in ALK-positive anaplastic large cell lymphoma. *Front Oncol*. 2022;12:815654.
- Marzec M, Halasa K, Liu X, et al. Malignant transformation of CD41 T lymphocytes mediated by oncogenic kinase NPM/ALK recapitulates IL-2-induced cell signaling and gene expression reprogramming. *J Immunol*. 2013;191:6200-6207.
- Lobello C, Tichy B, Bystry V, et al. STAT3 and TP53 mutations associate with poor prognosis in anaplastic large cell lymphoma. *Leukemia*. 2021;35(5):1500-1505.
- Crescenzo R, Abate F, Lasorsa E, et al. Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. *Cancer Cell*. 2015;27(4):516-532.
- Fitzpatrick MJ, Massoth LR, Marcus C, et al. JAK2 rearrangements are a recurrent alteration in CD30+ systemic T-cell lymphomas with anaplastic morphology. *Am J Surg Pathol*. 2021;45:895-904.
- Zhang Q, Raghunath PN, Vonderheid E, Odum N, Wasik MA. Lack of phosphotyrosine phosphatase SHP-1 expression in malignant T-cell lymphoma cells results from methylation of the SHP-1 promoter. *Am J Pathol*. 2000;157:1137-1146.
- Zhang Q, Wang HY, Liu X, Wasik MA. STAT5A is epigenetically silenced by the tyrosine kinase NPM1-ALK and acts as a tumor suppressor by reciprocally inhibiting NPM1-ALK expression. *Nat Med*. 2007;13:1341-1348.
- Zhang Q, Wang HY, Woetmann A, Raghunath PN, Odum N, Wasik MA. STAT3 induces transcription of the DNA methyltransferase 1 gene (DNMT1) in malignant T lymphocytes. *Blood*. 2006;108(3):1058-1064.
- Zhang Q, Wang HY, Liu X, Bhutani G, Kantekure K, Wasik M. IL-2R common  $\gamma$ -chain is epigenetically silenced by nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) and acts as a tumor suppressor

- by targeting NPM-ALK. *Proc Natl Acad Sci USA*. 2011;108:11977-11982.
19. Kasprzycka M, Marzec M, Liu X, et al. Nucleophosmin/anaplastic lymphoma kinase NPM-ALK oncoprotein induces T regulatory cell phenotype by activating STAT3. *Proc Natl Acad Sci USA*. 2006;103:9964-9969.
  20. Marzec M, Zhang Q, Goradia A, et al. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). *Proc Natl Acad Sci USA*. 2008;105:20852-20857.
  21. Hebart H, Lang P, Woessmann W. Nivolumab for refractory anaplastic large cell lymphoma: A case report. *Ann Intern Med*. 2016;165:607-608.
  22. Zhang Q, Wang H, Kantekure K, et al. Oncogenic tyrosine kinase NPM-ALK induces expression of the growth-promoting receptor ICOS. *Blood*. 2011;118:3062-3071.
  23. Marzec M, Liu X, Wong W, et al. Oncogenic kinase NPM/ALK induces expression of HIF1a mRNA. *Oncogene*. 2011;30:1372-1378.
  24. Zhang J, Wang P, Wu F, et al. Aberrant expression of the transcriptional factor Twist1 promotes invasiveness in ALK-positive anaplastic large cell lymphoma. *Cell Signal*. 2012;24:852-858.
  25. Gelebart P, Hegazy S, Wang P, et al. Aberrant expression and biological significance of Sox2, an embryonic stem cell transcriptional factor, in ALK-positive anaplastic large cell lymphoma. *Blood Cancer J*. 2012;2:e82.
  26. Wang P, Zhang JD, Wu F, et al. The expression and oncogenic effects of the embryonic stem cell marker SALL4 in ALK-positive anaplastic large cell lymphoma. *Cell Signal*. 2012;24:1955-1963.
  27. Lai R, Ingham RJ. The pathobiology of the oncogenic tyrosine kinase NPM-ALK: a brief update. *Ther Adv Hematol*. 2013;4:119-131.
  28. Pawlicki JM, Cookmeyer DL, Maseda D, et al. NPM/ALK-induced reprogramming of mature TCR-stimulated T cells results in dedifferentiation and malignant transformation. *Cancer Res*. 2021;81:3241-3254.
  29. Zhang Q, Wang HY, Nayak A, et al. Induction of transcriptional inhibitor HES1 and the related repression of tumor-suppressor TXNIP are important components of cell-transformation program imposed by oncogenic kinase NPM-ALK. *Am J Pathol*. In revision.
  30. Nadeu F, Delgado J, Royo C, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. *Blood*. 2016;127:2122-2130.
  31. Rossi D, Brusca A, Spina V, et al. Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness. *Blood*. 2011;118:6904-6908.
  32. Tausch E, Stilgenbauer S. BIRC3 mutations in chronic lymphocytic leukemia: uncommon and unfavorable. *Haematologica*. 2020;105:255-256.
  33. Wierda W.G., Rawstron A., Cymbalista F. et al. Measurable residual disease in chronic lymphocytic leukemia: expert review and consensus recommendations. *Leukemia*. 2021;35:3059-3072.
  34. Davids MS, Lamson BL, Tyekucheva S, et al. Acalabrutinib, venetoclax, and obinutuzumab as front-line treatment for chronic lymphocytic leukaemia: a single-arm, open-label, phase 2 study. *Lancet Oncol*. 2021;22:1391-1402.
  35. Gascoyne RD, Chan JKC, Campo E, et al. Diffuse large B-cell lymphoma, NOS. In Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (Eds): *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition)*. IARC: Lyon 2017. P291-297.
  36. Crump M, Neelapu SS, Farooq U, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. *Blood*. 2017;130:1800-1808.
  37. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403:503-511.
  38. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med*. 2002;346:1937-1947.
  39. Abramson JS. Hitting back at lymphoma: How do modern diagnostics identify high-risk diffuse large B-cell lymphoma subsets and alter treatment? *Cancer*. 2019;125:3111-3120.
  40. Roschewski M, Staudt LM, Wilson WH. Diffuse large B-cell lymphoma-treatment approaches in the molecular era. *Nature Rev Clin Oncol*. 2014;11:12-23.
  41. Schmitz R, Wright GW, Huang DW, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. *N Engl J Med*. 2018;378:1396-1407.

42. Younes A, Sehn LH, Johnson P, et al. Randomized Phase III Trial of Ibrutinib and Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone in Non-Germinal Center B-Cell Diffuse Large B-Cell Lymphoma. *J Clin Oncol*. 2019;37:1285-1295.
43. Wilson WH, Wright GW, Huang DW, et al. Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL. *Cancer Cell*. 2021;39:1643-1653.
44. Wilson WH, Young RM, Schmitz R, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nature Med*. 2015;21:922-926.
45. Susanibar-Adaniya S, Barta SK. 2021 Update on Diffuse large B cell lymphoma: A review of current data and potential applications on risk stratification and management. *Am J Hematol*. 2021;96:617-629.
46. Nowakowski GS, Czuczman MS. ABC, GCB, and Double-Hit Diffuse Large B-Cell Lymphoma: Does Subtype Make a Difference in Therapy Selection? *Am Soc Clin Oncol Educ Book*. 2015:e449-457.
47. Morin RD, Scott DW. DLBCL subclassification: divide and conquer? *Blood*. 2020;135:1722-1724.
48. Zhao MY, Auerbach A, D'Costa AM, et al. Phospho-p70S6K/p85S6K and cdc2/cdk1 are novel targets for diffuse large B-cell lymphoma combination therapy. *Clin Cancer Res*. 2009;15:1708-1720.
49. Dai B, Zhao XF, Mazan-Mamczarz K, et al. Functional and molecular interactions between ERK and CHK2 in diffuse large B-cell lymphoma. *Nature Commun*. 2011;2:1-9.
50. De Luca A, Maiello MR, D'Alessio A, Pergameno M, Normanno N. The RAS/RAF/MEK/ERK and the PI3K/AKT signalling pathways: role in cancer pathogenesis and implications for therapeutic approaches. *Expert Opin Ther Targets*. 2012;16 Suppl 2:S17-27.
51. Tasian SK, Teachey DT, Rheingold SR. Targeting the PI3K/mTOR pathway in pediatric hematologic malignancies. *Front Oncol*. 2014;4:1-8.
52. Zhao XF, Gartenhaus R. Phospho-p70S6K and cdc2/cdk1 as therapeutic targets for diffuse large B-cell lymphoma. *Expert Opin Ther Targets*. 2009;13:1085-1093.