tumor-associated macrophages from FL samples compared with reactive tonsils. This suggests that elements of the microenvironment may be organized to favor interactions with FL cells. The additional finding of DC-SIGN expression by FL lymphatic endothelial cells also hints at a potential mechanism for disease dissemination.

Both groups demonstrated heterogeneous binding of recombinant DC-SIGN to sIg on immunoglobulin M–positive (IgM^{+}) FL cells, with Amin et al noting DC-SIGN staining to be significantly higher on IgM[+] than IgG[+] FL samples. Linley et al suggest that there may be a positive correlation between sIgM expression and DC-SIGN binding, whereas a biochemical analysis by Amin et al also indicates that DC-SIGN high-binding IgM[+] FL might have a higher mannose glycosylation profile compared with DC-SIGN low-binding IgM[+] FL. Other mannose-binding lectins including endogenous mannose receptor and soluble lectins produced by opportunistic bacteria can also interact with FL sIg,[6,7] suggesting that lectin-mediated interactions may not be limited to DC-SIGN. Thus, although the nature of lectin binding to FL cells in vivo is likely multifaceted, the findings that DC-SIGN bound specifically to FL B cells but not nonmalignant residual B cells and bound poorly to normal naive, memory, or germinal center B cells underscore this interaction as a feature that is unique to the malignant cells.

Using fluorescence microscopy and flow cytometry, Amin et al subsequently showed that DC-SIGN binding to FL cells induced organization of the BCR and CD19 into signaling platforms in a manner that is similar to antigen-mediated BCR activation in normal B cells (see figure). This caused a delayed but sustained phosphorylation of key downstream BCR signaling kinases including spleen tyrosine kinase (SYK), protein kinase B (AKT), and extracellular signal-regulated kinase (ERK). Similarly, Linley et al found that in contrast to normal B cells, exposure of FL samples to DC-SIGN triggered prolonged phosphorylation of AKT, ERK, and phospholipase C-γ-2 (PLCγ2) and increased expression of cMYC, supporting the concept that DC-SIGN is able to activate and maintain proliferation signals that could promote disease progression.

In experiments carried out by both groups, DC-SIGN–mediated BCR signaling was attenuated with inhibitors of Bruton tyrosine kinase (BTK) or SYK, revealing the therapeutic potential of the lectin interaction. Indeed, Amin et al further demonstrated that coculture of in vitro–differentiated macrophages with FL cells led to observable cell-cell interactions with coclustering of DC-SIGN on macrophages and BCR on FL cells and that blockade of these interactions with an anti-DC-SIGN antibody reduced viability of FL cells in culture.

Although Linley et al did not identify any consistent differences in substrate phosphorylation between IgM[+] and IgG^{+} FL cells, Amin et al found that BCR activation in DC-SIGN high-binding IgM[+] FL was stronger than in DC-SIGN low-binding IgM[+] FL and that IgG^{+} FL cells were poorly activated by DC-SIGN. These discrepancies may be due to the limited number of cases analyzed as well as technical variations in the activation conditions. Nonetheless, the collective findings support a model whereby lectins within the microenvironment might foster FL cell survival and proliferation by providing a continuous activation signal through antigen-independent interactions with sIg (see figure). These studies provide a foundation for future investigations directed at understanding the role of mannosylated sIg in lymphomagenesis, determining the clinical significance of mannose glycosylation profiles in FL, and exploring targeted therapeutic approaches to disrupt glycan-lectin interactions in the microenvironment.

Conflict-of-interest disclosure: The author declares no competing financial interests. ∎

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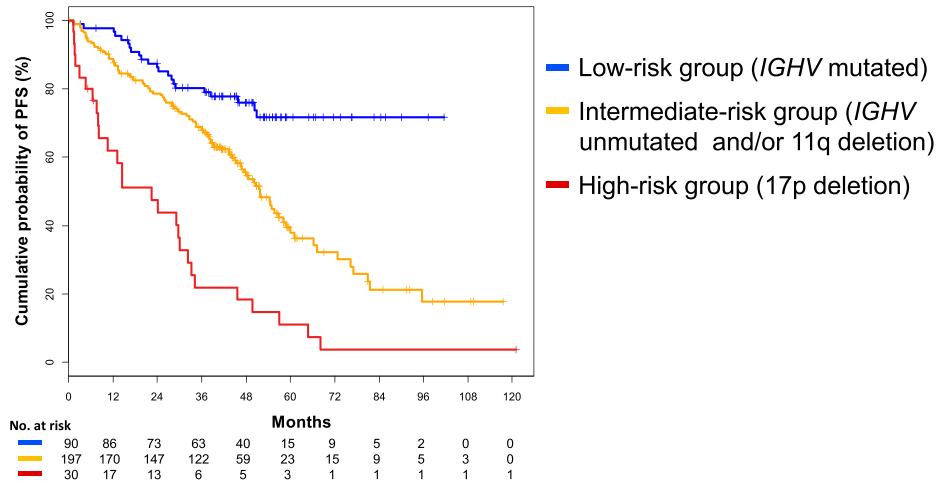
● ● ● **LYMPHOID NEOPLASIA**

Comment on Rossi et al, page 1921

So FCR, so good

Claire Dearden   THE ROYAL MARSDEN HOSPITAL AND BIOMEDICAL RESEARCH CENTRE

In this issue of *Blood*, Rossi et al show that certain predictive biomarkers are able to identify a subgroup of patients with chronic lymphocytic leukemia (CLL) who have an exceptionally good outcome following front-line therapy with the combination of fludarabine, cyclophosphamide, and rituximab (FCR).[1]

Fifty years ago, there was only one therapy, chlorambucil, that was commonly used to treat CLL. The treatment landscape has undergone dramatic change since then, particularly in the last 3 years, and there is now a bewildering array of available agents, both chemotherapy and targeted nonchemotherapy drugs, used alone and

	Events	Total	Median PFS	95% CI
(blue)	22	90	nr	na
(yellow)	102	197	51.7	46.1-57.2
(red)	27	30	22.5	8.5-36.4

Pairwise comparisons of the PFS curves

p			
(blue)	-	0.0001	<0.0001
(yellow)	0.0001	-	<0.0001
(red)	<0.0001	<0.0001	-

Legend:
- Low-risk group (*IGHV* mutated)
- Intermediate-risk group (*IGHV* unmutated and/or 11q deletion)
- High-risk group (17p deletion)

No. at risk (Months: 0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120)

(blue)	90	86	73	63	40	15	9	5	2	0	0
(yellow)	197	170	147	122	90	59	23	15	9	3	0
(red)	30	17	13	6	5	3	1	1	1	1	1

Estimates of PFS for a group of 404 patients with CLL divided into 3 risk groups: high risk (in red), 17pdel; intermediate risk (in yellow), unmutated *IGHV* and/or 11qdel but not 17pdel; low risk (in blue), mutated *IGHV* and no 11q or 17pdel. See Figure 1A in the article by Rossi et al that begins on page 1921.

in various combinations. A number of retrospective studies and prospective clinical trials over the last 15 years have sought to determine clinical and biological parameters that can identify those patients who will respond well to a particular treatment and conversely those patients who will have a worse outcome. There has been some success in this endeavor to "personalize" the approach to treatment. Döhner et al[2] first described the hierarchical model of cytogenetic aberrations highlighting the poor outcome for patients with deletions of 17p and 11q. Hamblin et al[3] demonstrated the difference in survival between patients with mutated vs unmutated immunoglobulin heavy chain genes (*IGHV*). These parameters have now been studied prospectively in several clinical trials, confirming the predictive value of these tests, which are readily available for patients who are embarking on their first treatment of CLL. Indeed, the strong prediction for poor response to conventional treatment in those patients whose CLL cells harbor abnormal *TP53* (deletion or mutation) on chromosome 17 has led to the introduction of novel tailored approaches for these individuals, including early stem cell transplantation. There also continue to be concerns related to the immediate and delayed toxicities associated with the use of conventional myelo-suppressive and DNA-damaging chemotherapy. The advent of newer targeted nonchemotherapy drugs is attractive in this regard and may have application across a much wider range of CLL patients, including those with adverse cytogenetics and those not fit enough to

receive more intensive chemotherapy. It is therefore of paramount importance that robust data are available to aid decision-making related to the choice of initial therapy for patients with CLL.

Since the initial reports from the MD Anderson Cancer Center on the efficacy of FCR,[4] this has been adopted internationally as the "gold standard" treatment of younger fit patients with CLL. With extended follow-up from this initial study, together with data from the German CLL study group trial (CLL8) of FCR vs FC, it has become clear that a subset of patients can be identified who are likely to have an exceptionally good outcome.[5-7] This subgroup is defined by the presence of mutated *IGHV* genes together with a lack of adverse cytogenetic features, notably deletions of 17p and 11q.

Rossi et al retrospectively studied a large cohort of 404 Italian patients who received front-line treatment with FCR and had biological parameters assessed at baseline.[1] They confirm the finding that the 28% of patients in this cohort with mutated *IGHV* and no 17p or 11q deletions have very prolonged progression-free survival (PFS; median not reached, 71% at 5 years) and overall survival (OS; 91% at 5 years). As with the other studies from MD Anderson Cancer Center and CLL8, the risk of relapse appears to decline significantly after 4 years, with a plateau possibly emerging on the curve (see figure). A small number of the patients were tested for minimal residual disease (MRD) in peripheral blood after more than 6 years in remission and found to be MRD negative. Importantly, they show that this group of patients has a survival similar to that of an

age-matched general Italian population without CLL, suggesting that neither the CLL itself nor the treatment received affected survival in this good-risk group of patients. However, it is important to note that in the study by Rossi et al, the follow-up remains relatively short, with little data beyond 5 years. More extended follow-up of this patient cohort is therefore needed to confirm the findings.

Published data on front-line therapy with novel agents such as ibrutinib and idelalisib are still limited to very small numbers of patients with relatively short follow-up.[8] Despite the extraordinarily good results (96% PFS at 30 months for front-line ibrutinib), there remain issues around duration of and compliance with treatment, short- and long-term tolerability, emergence of resistance, and cost. It would be naïve to imagine that all patients with CLL will benefit equally from novel therapies in the long term without any complications or progression. It is therefore crucial that the necessary clinical trials are undertaken to establish exactly which patient is likely to benefit most from the different therapeutic approaches to allow selection of a tailored patient-specific treatment plan. Unlike the trials in relapsed/refractory CLL, these studies will take time to conduct and to reach end points such as PFS and OS. It will thus be many years before there are answers to these important questions. In the meantime, there is now evidence from 3 large patient cohorts including more than 1000 patients treated with first-line FCR, some with long follow-up, that those patients with mutated *IGHV*, no adverse cytogenetics, and of the right age and fitness to receive

combination chemo-immunotherapy can expect to achieve very durable remissions and near-normal survival. The small number of patients who fall into this category may thus be able to return to normal life and work after a single 6-month, cost-effective treatment without the need for ongoing medication and with less uncertainty about their longer-term prospects.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● ● **MYELOID NEOPLASIA**

Targeting leukemia's "fatty tooth"

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In this issue of Blood, Ricciardi et al report a novel fatty acid oxidation (FAO) inhibitor, ST1326, that effectively inhibits proliferation, survival, and chemoresistance in leukemia cell lines and primary samples.[1]

It has been suggested that FAO promotes leukemia stem cell survival and quiescence by supporting mitochondrial oxidative metabolism and increasing the threshold for activation of the intrinsic apoptotic pathway.[2] Metabolically, FAO feeds large amounts of fatty acyl–derived acetyl-coenzyme A (CoA) into the Krebs cycle, allowing the regeneration of citrate and the continuous production of nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide to support the molecular reduction of oxygen into water (see figure). The regeneration of citrate is an obligate step for the de novo synthesis of lipid membrane components; it is essential for cellular proliferation and forms part of an apparently "futile" metabolic cycle of FAO and fatty acid synthesis that has been proposed to be another key hallmark of cancer cell metabolism.[3] Although seemingly wasteful, this futile cycle is essential for the generation of antioxidant defenses[3] and antagonizes the oligomerization of Bax and Bak in response to apoptotic stimuli,[2] providing a fundamental barrier to cell death. Because FAO occurs in the mitochondrial matrix, which is mostly impermeable to free fatty acids or CoA esterified fatty acids, the rate-limiting step is the transfer of acyls from CoA to carnitine by the enzyme carnitine palmitoyltransferase 1 (CPT1), producing acylcarnitines. Acylcarnitines are then spontaneously translocated through the mitochondrial membrane into the matrix, where CPT2 catalyzes the re-formation of CoA esterified fatty acids to be metabolized by FAO.

ST1326 is an aminocarnitine derivative that is highly selective for the CPT1a isoform,[4] a favorable characteristic when compared with etomoxir—the prototypical CPT1 inhibitor that irreversibly inhibits both a (liver) and b (muscle/heart) isoforms of the enzyme.[5] Although etomoxir has been reported to efficiently induce apoptosis and sensitize leukemia cells to chemotherapy,[2] its high cost and the potential on-target toxicity of inhibiting FAO in skeletal and cardiac muscle[6,7] have precluded its clinical development as an antileukemic agent. In contrast, an oral formulation of ST1326 (teglicar) has been tested in phase 2 studies for the treatment of type 2 diabetes, in which it demonstrated an excellent safety profile. Importantly, Ricciardi et al demonstrate for the first time that (1) all leukemia cell lines and primary acute myeloid leukemia cells examined express the CPT1a isoform of the enzyme; (2) ST1326 potently inhibits FAO and overall mitochondrial oxygen consumption in cell lines and primary samples; and (3) ST1326 induces cell death in disease initiating but not normal bone marrow progenitors. Taken together, these findings suggest that ST1326 may be a targeted agent for the treatment of leukemia.

Mechanistically, ST1326 induces cytotoxicity via activation of the mitochondrial apoptotic pathway, as evidenced by an early drop in mitochondrial membrane potential ($\Delta\Psi_m$) that precedes the externalization of phosphatidyl serine and DNA fragmentation. Intriguingly, although ST1326 has been reported to be a competitive/reversible inhibitor of CPT1a,[8] drug washout experiments revealed that this agent was irreversibly cytotoxic, suggesting that even transient inhibition of CPT1a-dependent FAO results in a lethal mitochondrial insult in leukemia cells. Although the precise nature of this insult is not discussed by Ricciardi et al, the authors convincingly demonstrate a marked (>6-fold) accumulation of palmitate in the cytoplasm of leukemia cells treated with ST1326, a critical finding that supports the notion that excess free fatty acids perturb mitochondrial membrane integrity and/or function, at least in part by virtue of their detergent properties that may promote the leakage of protons into the mitochondrial matrix, "short-circuiting" $\Delta\Psi_m$ (see figure). In addition, it is tempting to speculate that, like etomoxir[2] and the recently reported FAO inhibitor avocatin B,[9] ST1326 may induce accumulation of ROS and/or may facilitate Bax/Bak oligomerization in the outer