future. However, a note of caution is that many potential therapies have been shown to have *in vivo* efficacy in AML, but when tested clinically have had little or no effect on this disease.

In conclusion, by targeting a number of different oncogenic pathways, *in vitro* and *in vivo* treatment with ARQ531 results in reduced AML cell viability, reduced tumor growth and improved survival of animals. The research by Soncini *et al.* suggests that a multi-targeted inhibitor such as ARQ531 is required to impair AML survival effectively; since this drug does not rely specifically on high expression of BTK or other tyrosine kinases it could be widely applicable to different subtypes of AML.

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A step ahead toward precision medicine for chronic lymphocytic leukemia

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The concept of precision medicine applied to human tumors implies the personalized tailoring of clinical management and treatment choices according to the status of an array of molecular biomarkers, in conjunction with other patient features.[1] In chronic lymphocytic leukemia (CLL), the extensive body of genetic data that have been accumulated in recent years has led to the identification of many new molecular biomarkers with prognostic value. However, only a few of these serve the role of true predictors for choosing the most appropriate treatment for any given patient.[1,2] The active search for molecular predictors in CLL is becoming increasingly more important in the current therapeutic landscape of the disease, that ranges from chemo-immunotherapy with both old and newer monoclonal antibodies (mAb) to chemo-free options based on B-cell receptor (BCR) inhibitors, targeting either Bruton tyrosine kinase or phosphatidyilinositol-3-kinase, and BCL2 inhibitors.[3,4]

In this issue of *Haematologica*, Tausch *et al.* have analyzed the prognostic and, more importantly, the predictive role of a panel of gene mutations in the randomized, phase III COMPLEMENT1 trial comparing chlorambucil with ofatumumab-chlorambucil in treatment-naïve CLL patients not eligible for intensive therapy because of age or comorbidities.[5] The COMPLEMENT 1 trial had documented that addition of the type 1 anti-CD20 mAb ofatumumab to chlorambucil leads to clinically significant improvement in progression-free survival (PFS) (22.4 months in the arm treated with ofatumumab chlorambucil *vs*. 13.1 months in the arm treated with single agent chlorambucil), with a manageable side effect profile.[6] But whether ofatumumab provided an advantage to all molecular subgroups of CLL remains unexplored. Remarkably, in the genetic analysis performed by Tausch *et al.*, mutations of *NOTCH1* were seen to predict weak benefit from the addition of ofatumumab to the chlorambucil backbone.[5] The *NOTCH1* signaling pathway is a key feature in CLL growth and survival, and is deregulated by mutations in a sizable fraction of CLL[7] (Figure 1). *NOTCH1* mutations in CLL may target either the autoregulatory PEST domain, or the non-coding 3'-untranslated region (3'-UTR) sequence.[7] In the context of

the COMPLEMENT1 trial, the addition of ofatumumab to chlorambucil provided a significant benefit in PFS to *NOTCH1* wild-type patients, whereas no statistically significant benefit was achieved in *NOTCH1* mutated cases, including patients whose mutations disrupted the *NOTCH1* PEST autoregulatory domain as well as patients with *NOTCH1* mutations affecting the 3'-UTR of the gene.[5]

The refractoriness to ofatumumab imparted by *NOTCH1* mutations is reminiscent of the refractoriness to another type 1 anti-CD20 mAb, namely rituximab, that had been observed in the CLL8 trial comparing fludarabine-cyclophosphamide with fludarabine-cyclophosphamide-rituximab (FCR) in young and fit CLL patients.[8] In fact, in the CLL8 trial, rituximab failed to improve response and survival in patients carrying *NOTCH1* mutations.[8] The fact that *NOTCH1* mutations behave as a predictor of reduced benefit from type 1 anti-CD20 mAb in two prospective, randomized trials with different anti-CD20 antibodies (ofatumumab in COMPLENT1; rituximab in CLL8), different chemotherapy backbones (chlorambucil in COMPLEMENT1; fludarabine-cyclophosmide in CLL8), and different target CLL populations (patients not eligible to intensive therapy in COMPLEMENT1; patients eligible to fludarabine-containing regimens in CLL8) contributes further to the robustness of the predictive significance of *NOTCH1* mutations in CLL treated with chemo-immunotherapy containing anti-CD20 type 1 mAb.[5,8]

The obvious question is whether the novel, type 2 anti-CD20 mAb in use for CLL, namely obinutuzumab, may overcome the refractoriness imparted by *NOTCH1* muta-

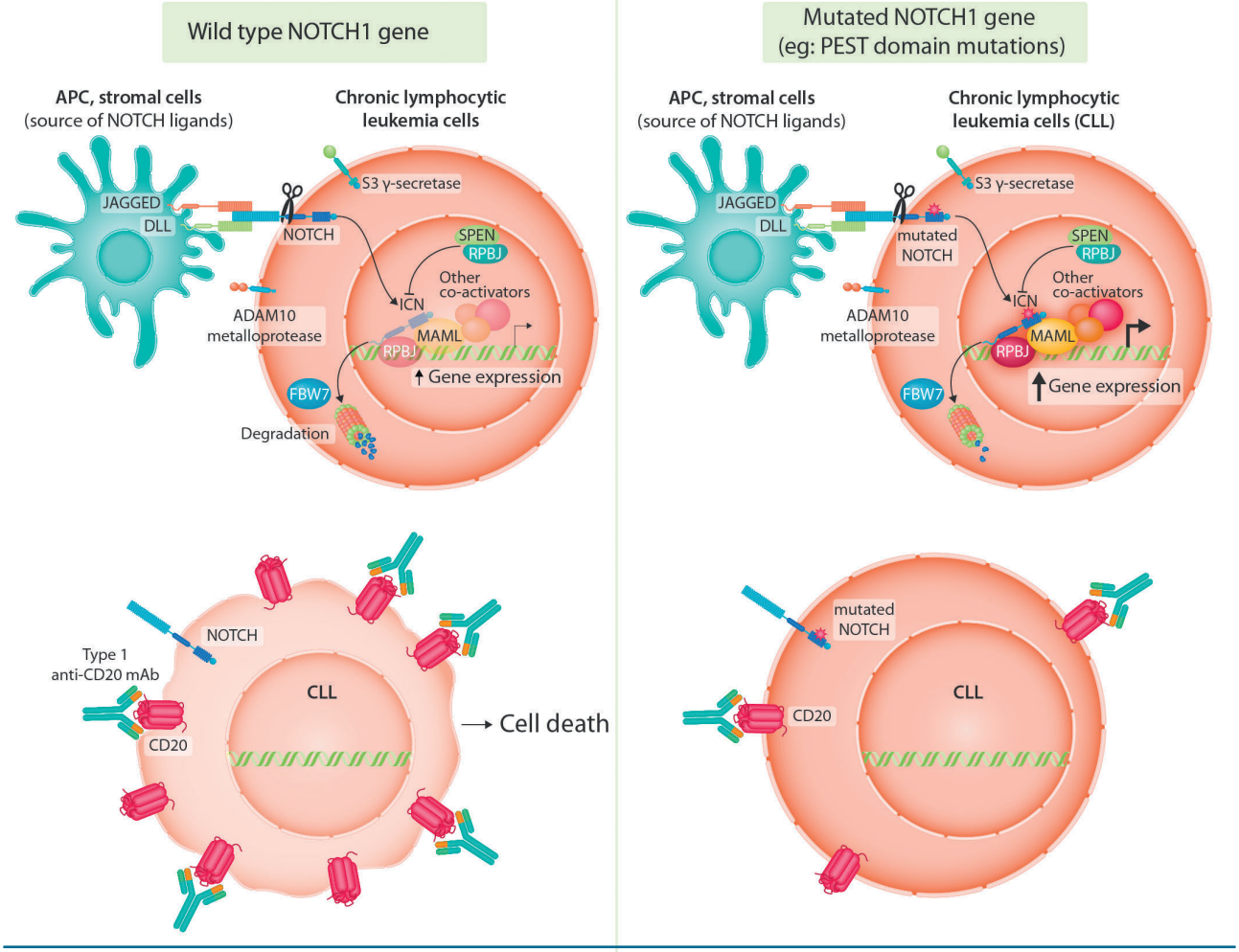

Figure 1. *NOTCH1* signaling pathway and effects of *NOTCH1* mutations on CLL susceptibility to anti-CD20 mAb. In the context of a wild-type *NOTCH1* gene (left panel), ligands (DLL -1, -3, -4 belonging to the Delta-like family or JAGGED -1, -2 belonging to the Serrate family) expressed by stromal cells and by antigen presenting cells (APC) bind to the extracellular portion of the NOTCH1 receptor on CLL cells. Ligand-receptor binding triggers sequential cleavages of the NOTCH1 receptor mediated by the ADAM10 metalloprotease and the S3 γ-secretase. As a consequence, the IntraCellular NOTCH1 (ICN) domain is free to translocate to the nucleus, where it interacts with RBPJ and other co-activators to induce transcription of target genes promoting cell growth and survival and other cellular programs. The signaling cascade is terminated by ubiquitinylation of the NOTCH1 autoregulatory PEST domain, that is mediated by the FBW7 complex and leads to ICN degradation in the proteasome. In CLL cells with wild type NOTCH1 genes, type 1 anti-CD20 antibodies (rituximab, ofatumumab) induce cell death *in vitro* and, *in vivo* contribute to better patient outcomes in patients treated with chemo-immunotherapy. *NOTCH-1* mutations occur in a sizeable fraction of CLL (right panel), upregulate NOTCH1 signaling and lead to increased expression of target genes. Most mutations in CLL disrupt the PEST domain, reducing proteasomal degradation of ICN and stabilizing ligand-triggered *NOTCH1* signaling. Type 1 anti-CD20 mAb are less efficacious against *NOTCH1* mutated CLL cells both *in vivo* and *in vitro*. The exact mechanism of anti-CD20 refractoriness associated with *NOTCH1* mutations is not fully understood, but has been suggested to be linked, at least in part, to downregulation of CD20 expression.

tions to anti-CD20 therapy. This may be possible, since the glycoengineered type 2 anti-CD20 obinutuzumab exploits a different mode of action, based on enhanced antibody-dependent cell-mediated cytotoxicity and increased direct cell death compared to the type 1 anti-CD20 mAb rituximab and ofatumumab.[9] Preliminary data seem to suggest that obinutuzumab might be able to overcome such refractoriness in the CLL11 trial comparing obinutuzumab-chlorambucil with rituximab-chlorambucil.[10]

Guidelines for CLL still recommend chemo-immunotherapy as a therapeutic option despite the advent of BCR and BCL2 inhibitors.[11] In this context, knowledge of *NOTCH1* mutation status might be important in clinical decision-making whenever a chemo-immunotherapy regimen containing an anti-CD20 mAb is being offered to patients. The evidence acquired so far on anti-CD20 refractoriness and *NOTCH1* mutations would support the concept that, in the presence of a mutated *NOTCH1* gene, the use of a chemo-immunotherapy regimen containing a type 1 anti-CD20 mAb may not be the most appropriate choice and might be replaced by one of the many other therapeutic options that are currently available for CLL.[5,7,11] Recommendations by guidelines on this specific issue are desirable at this stage.

The use of anti-CD20 mAbs in CLL is not limited to chemo-immunotherapy regimens both in treatment-naïve and in relapsed/refractory patients. For example, the MURANO trial has shown the superiority of venetoclax-rituximab compared to bendamustine-rituximab in relapsed/refractory CLL.[12] The CLL14 trial has documented that venetoclax-obinutuzumab associates with longer PFS compared to chlorambucil-obinutuzumab in treatment-naïve CLL.[13] The iLLUMINATE trial has shown the advantage of ibrutinib-obinutuzumab over chlorambucil obinutuzumab as first-line therapy.[14] Ibrutinib-rituximab is superior to chemo-immunotherapy in an Eastern Cooperative Oncology Group (ECOG) trial devoted to treatment-naïve CLL.[15] At present, it is not known whether the reduced efficacy of type 1 anti-CD20 mAbs observed in *NOTCH1* mutated patients treated with chemo-immunotherapy would also be a feature of novel chemo-free regimens based on BCR or BCL2 inhibitors in combination with an anti-CD20 mAb.

The precise molecular mechanism through which *NOTCH1* mutations confer resistance to anti-CD20 type 1 mAb remains, to a certain extent, elusive (Figure 1). Though the biological relationship between NOTCH1 mutation expression and CD20 cell surface expression was not a specific focus of the report by Tausch *et al.*, measuring CD20 levels by flow cytometry in the COMPLEMENT1 trial population failed to reveal differences between *NOTCH1* mutated and wild-type cases.[5] Conversely, in a wide CLL series of almost 700 cases, CLL cells from cases harboring mutations of the *NOTCH1* PEST domain showed lower CD20 expression compared to *NOTCH1* wild-type cases.[16] Reduced surface expression of CD20 appears to be a feature also of CLL cases harboring a different type of *NOTCH1* mutations affecting the 3'-UTR of the gene.[17] Lower CD20 expression on the cell surface of CLL cells has been shown to be coupled to lower mRNA levels of the *MS4A1* gene that encodes the CD20 antigen.[16] As a consequence, cell lysis induced by anti-CD20 type 1 antibodies, namely rituximab and ofatumumab, appears to be also lower in *NOTCH1* mutated cases compared to CLL without this genetic lesion.[16] Consistent with these observations, pharmacological inhibition of the NOTCH1 protein or siRNA silencing of the *NOTCH1* gene have been shown to induce upregulation of the CD20 molecule on CLL cells.[16] It is well known that several epigenetic and transcription factors regulate expression of the *MS4A1* gene and of the CD20 antigen.[18] Interestingly, mutations of the *NOTCH1* intracellular domain lead to accumulation of mutated *NOTCH1* in the nucleus and may alter the fine epigenetic regulation of MS4A1 and CD20 expression through interactions with the RBPJ transcription factor that is involved in the *NOTCH1* signaling pathway.[16,18]

Overall, the biological relationship between *NOTCH1* signaling, its deregulation by mutations and expression of CD20 requires further investigation, ideally in study designs aimed at comparing different type 1 and type 2 anti-CD20 mAb in order to understand not only the mechanisms of resistance, but also the strategies to overcome such refractoriness. It should also be considered that *NOTCH1* belongs to a molecular pathway and that mutations in B-cell malignancies may also target other players of the pathway.[6] Because these genetic alterations either potentiate positive signals or compromise negative regulators of *NOTCH1*, it would be interesting to understand whether alterations of other *NOTCH1* pathway genes, in addition to *NOTCH1* itself, might have an effect on anti-CD20 mAb response *in vitro* and *in vivo*.

The clinical management and therapeutic landscape of CLL have changed substantially over the last few years and continue to evolve. The availability of a variety of treatment options, ranging from chemo-immunotherapy to molecular inhibitors of the BCR and BCL2 pathways, has generated the need to search for robust biomarkers that may assist clinicians in choosing the most suitable and sustainable treatment strategy for every patient. Guidelines recommend *TP53* disruption and IGHV mutation status as molecular predictors and these are commonly used when choosing treatment.[19] Tausch *et al.* now consolidate *NOTCH1* mutation status as a novel potential biomarker for optimizing anti-CD20 treatment when a chemo-immunotherapy option is offered to patients.[5] Other predictive biomarkers are also emerging, and include loss of function mutations of *BIRC3*, that deregulate the NFkB pathway and confer resistance or reduced efficacy with chemo-immunotherapy regimens,[20,21] as well as use of specific stereotyped BCR subsets, in particular subset #2, as observed in the correlative analysis of multicentric clinical trials.[22]

Step by step, precision medicine is becoming a solid reality in the field of CLL for the benefit of patients and to optimize allocation of resources in clinical practice. At present, the available therapeutic options for CLL that are recommended by guidelines have not always been subjected to rigorous and multiple head-to-head prospective comparisons, thus leaving several unanswered questions when physicians and patients need to make a treatment choice. Choosing wisely, based on robust molecular predictors, coupled to the patient's fitness and comorbidities,

might represent a viable and clinically meaningful strategy for achieving the best therapeutic outcome for the individual patient and to satisfy the need to optimize resources for the patient community.

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From weakly adhesive to highly thrombogenic: the shear gradient switch

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Formation of a blood clot within an artery, is a complex process orchestrated by numerous chemical and physical factors, including: platelets, endothelium, subendothelial matrix, soluble blood proteins involved in hemostasis e.g., fibrinogen and von Willebrand factor (VWF) and blood flow.[1] The pivotal role of flow characteristics in thrombosis and hemostasis has been well recognized in the field, as blood flow regulates the physical environment of the clotting process and the transport of molecules and blood cells.[2,3] More specifically, *in vivo* and *in vitro* studies under constant flow highlighted wall shear rate, the spatial rate of change in velocity near the wall which affects transport and friction forces near the wall, as a key parameter controlling the thrombosis processes.[4–7] Under physiological conditions, wall shear is tightly regulated in the arterial vascular system. However, under pathological conditions, such as arterial stenosis, wall shear rate can increase significantly above its physiological level.[8] Thus, the study of thrombosis under pathological high wall shear rates has received considerable attention and has uncovered important shear dependent processes such as platelet shear activation and VWF unfolding.[9,10] However, unlike constant wall shear conditions, in stenotic sites the flow is complex and the wall shear rate changes dramatically at the flow acceleration and deceleration zones.[11] Several studies have investigated platelet aggregation mechanisms under complex shear gradient to emphasize the key role of disturbed hemodynamics in thrombus cascade.[12] One important study in this field was conducted by Nesbitt *et al.*, Nature