Prognostic factors in chronic lymphocytic leukaemia (CLL)

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Abstract

Many prognostic factors have been identified in CLL. Based on assessment of BCR structure and function, a subdivision is possible into subtypes (IGHV unmutated and mutated, V3-21 usage) with distinct biological and clinical characteristics. Recurrent genomic aberrations (i.e. 11q and 17p deletion) and gene mutations (*TP53*, *ATM*) help to define biological and clinical subgroups. In addition, serum markers (e.g. TK, ß2-MG), cellular markers (e.g. CD38, ZAP70) and clinical staging impacts outcome in CLL. The biological characterisation of CLL has not only led to progress in outcome prediction but also has begun to be translated into novel treatment strategies. Nonetheless most factors associated with prognosis have not been thoroughly interrogated for their predictive value in the light of different therapeutic approaches. With a growing number of agents acting on specific biological targets and used in different clinical situations, the future is likely to bring the identification of predictive factors in CLL.

Introduction

Some patients with CLL survive for many years or decades without need for treatment. Others have a rapidly fatal disease despite therapy. This heterogeneity is only in part accounted for by the staging systems of Rai and Binet which classify patients according to tumor burden and haematopoietic impairment. Within the past decade(s) – similar to many other cancers - CLL has been shown to be a biologically diverse disorder(1-3). The diverse biological pathomechanisms explain much of the clinical heterogeneity. Many of these biological subgroups define prognostic factors, but shortcomings and room for stratification remain. Future approaches should focus on the definition of the clinical use of prognostic factors based on prospective trial data and the identification of predictive factors, which will lead to an individualized approach to the treatment of CLL. This review will summarize the most important prognostic factors in CLL and focus on the developing field of predictive factors.

Prognostic and predictive factors

The most important prognostic factors in CLL are clinical stage, markers of tumour load (e.g. TK, ß2MG), cellular protein expression (e.g. CD38, ZAP70), and genetic parameters including IGHV mutational status, genomic aberrations and individual gene mutations(3-6, 7). There is a significant hierarchy in these prognostic factors(8, 9)(Fig. 1).

While it is important to have prognostic markers, it is also critical to account for the difference between prognostic and predictive markers. A prognostic factor is a situation, condition or characteristic of a patient that can be used to estimate outcome due to the disease irrespective of the treatment given. The important distinction to a predictive factor is the treatment response variable. A predictive factor is a condition or finding that predicts the differential efficacy of a particular therapy based on marker status (i.e. patients with presence or absence of a specific marker will respond differently to a specific treatment)(10, 11). Most

factors associated with prognosis in CLL have not yet convincingly been shown to demonstrate predictive impact.

CLINICAL STAGE ACCORDING TO BINET AND RAI

The standard clinical procedures to estimate prognosis in CLL are the staging systems developed by Rai and Binet(12, 13). These systems rely on physical examination and a blood count to detect lymphadenopathy, organomegaly, platelet and haemoglobin values. The prognostic impact of these staging systems was confirmed in many independent studies. However, there is still heterogeneity in the course of the disease of patients within a single stage group. There is currently little evidence that clinical stage can be used as a predictive factor in CLL.

MARKERS OF TUMOR BURDEN

In addition to the clinical staging systems, other “simple” parameters of disease activity and tumor burden such as the lymphocyte count, the lymphocyte doubling time, the serum LDH level or the bone marrow infiltration pattern were shown to be of prognostic relevance in CLL (reviewed in (14)). Elevated LDH levels and high lymphocyte counts are associated with disease activity. Although correlated with clinical stage, the lymphocyte doubling time was shown to have a prognostic significance by itself: lymphocyte doubling time of 12 months or less identifies a population of patients with poor prognosis(15). The lymphocyte infiltration of the bone marrow has been evaluated for its impact on prognosis. Several studies showed that cases with diffuse bone marrow infiltration had a poor prognosis as compared with cases presenting with a nodular pattern(16). More recent analyses suggest that BM infiltration

pattern may not be an independent prognostic factor adding to current models when important genetic markers are considered(17).

A number of serologic parameters such as thymidine kinase (TK) and ß2-microglobulin (ß2-MG) have been shown to provide information on outcome(4, 7, 18). TK levels correlate with the proliferative activity of CLL cells and elevated levels predict disease progression.

Beta2-microglobulin (ß2-MG) is an extracellular protein associated with the class I major histocompatibility complex. Its serum levels show a correlation with the clinical staging systems. Recently, a prognostic normogram based on a retrospective analysis from the MD Anderson Cancer Center has been developed including age, beta-2 microglobulin, absolute lymphocyte count, sex, Rai stage, and number of involved lymph node groups(4).

All of the above factors summarized as measures of tumor burden can be grouped within the armamentarium of prognostic factors. There is however currently little data to suggest that they have predictive properties, which could be used to guide treatment decisions. This appears to be particularly the case for TK and ß2-MG which confer prognostic information in early stage, advanced stage (at first line treatment) as well as refractory CLL and irrespective of the treatment given (4, 17, 19)(Fig. 2). The prognostic categorisation based on TK appears to require adjusting of cut-off depending on the clinical situation, while ß2-MG cut-off appears more stable in different disease stages.

B CELL RECEPTOR, IGHV MUTATIONAL STATUS

B cell response to antigen is mediated through the B cell receptor (BCR) in normal and malignant B cells. B cells display distinct BCRs based on variable combination of V, D, J segments (heavy chain) and V, J gene segments (the light chain)(20). The repertoire is greatly

increased by the introduction of somatic hypermutation in the germinal centre reaction (or T cell independent hypermutation outside the germinal center)(20). CLL cases can be classified based on the degree of somatic hypermutation: those with mutated immunoglobulin heavy chain variable gene segments (IGHV) and those with unmutated IGHV genes(21-23). While the cut-off underlying this separation with < / > 98% sequence identity is arbitrary, the groups have a profoundly different clinical course(9, 21, 22). IGHV mutation status is clinically relevant as it predicts risk of disease progression and outcome in untreated patients with CLL(9, 21, 22). While CLL with unmutated IGHV follows an unfavorable course with rapid progression and earlier death, CLL with mutated IGHV often shows slow progression and long survival. IGHV mutation status is of prognostic importance in unselected patient cohorts, after treatment, as well as early stage (Binet A) patients (Fig. 1). Clinical trial data confirms the impact of IGHV mutation on outcome (24-27). The value of IGHV status to predict response to therapy has not been fully investigated. Overall response rate and particularly the response duration is worse in patients expressing this marker but there is little evidence for its use as a predictive marker in standard treatment regimens(26, 27).

There are important biological differences between the two (IGHV mutated and unmutated) groups. Unmutated IGHV CLLs have higher levels of the protein tyrosine kinase ζ-associated protein 70 (ZAP70) and CD38. Unmutated IGHV CLL are more likely to activate key signalling pathways in response to BCR activation (e.g. LYN, NFAT, AKT, SYK, ERK) .Upon BCR stimulation in CLL, key molecules of the BCR signalling cascade (e.g. Syk and ZAP-70) are recruited and phosphorylated.(reviewed by Kipps(28)). The goups also demonstrate a different mRNA expression profile(29-31). IGHV unmutated CLL has greater proliferative capacity and shorter telomere length(32-34).

A high proportion of IGHV unmutated CLL cases carry stereotyped rearrangements of the IgVH, D and JH segments with very similar complementarity determining region (CDR) 3

regions. Overall, more than 20% of CLL patients carry these stereotypic BCRs(35-37). These findings suggest that common antigen(s) are recognized by malignant CLL cells. Stereotyped BCR are less likely to occur in IGHV mutated CLL. Specific V-genes in the VDJ rearrangement appear to be associated with distinct biological and clinical features (e.g. VH3-21)(38).

While the study of the IGHV mutational status has been pivotal to the understanding of CLL biology and our ability to separate prognostic subgroups in CLL, the role of the IGHV mutational status in guiding therapy is currently unresolved. Treatment decisions should not be based on IGHV status outside trials(24, 27, 39). Future strategies may evolve targeting deregulated signaling pathways of IGHV unmutated or mutated CLL cases (28, 40). In a setting where different BCR signaling components could be targeted, the IGHV mutation status may well turn out to become an important predictive marker.

SURROGATE MARKERS

As the determination of IGHV mutation status may not be practicable in all laboratories, a search for “surrogate markers” (parameters that are strongly correlated with IGHV mutation status) is ongoing. ZAP70, which was identified based on gene expression profiling studies, is most widely used. ZAP70 is a molecule usually involved in T-cell receptor signalling which is aberrantly expressed in some CLL cases. ZAP70 levels as measured by FACS separate distinct prognostic groups of Binet A CLL patients with similar clinical behaviour and great overlap to the groups segregated by IGHV mutation profile(41). In all patients in whom at least 20 percent of the leukemic cells were positive for ZAP-70, IgVH was unmutated, whereas IgVH mutations were found in 21 of 24 patients in whom less than 20 percent of the

leukemic cells were positive for ZAP-70. The prognostic impact of ZAP-70 erxpression has been confirmed in many studies(42, Rassenti, 2008 #324, 43, 44).

As with any prognostic marker, the relation of new prognostic markers must be related to other markers known to impact on outcome to define its independence or dependence of other factors. A number of studies have shown a strong association of high ZAP-70 expression and unmutated IGHV genes and BCR function(43-45). However, discordance of ZAP-70 expression and IGHV mutation status occurred in up to 25% of cases(44). The proportion of discordant cases may be particularly high in the distinct subgroups with V3-21 usage and 17p or 11q deletion (39%). The IGHV mutation status, V3-21 usage, the presence of high-risk genomic aberrations, but not ZAP-70 expression were identified as independent prognostic factors. Nonetheless, in this study the association of ZAP-70 and the IGHV mutation status was strong in cases without additional genetic high-risk features, and the majority of V3-21 cases showed high ZAP-70 expression irrespective of the IGHV mutation status. In the absence of high-risk genomic aberrations the IGHV status and ZAP-70 status may have similar prognostic impact, and might therefore be alternatively applied. A problem concerning ZAP70 determination in clinical practice is the challenge in the standardization of a FACS assay for its measurement. data suggest that ZAP70 methylation or mRNA expression may be used as potentially more robust methods of assessing ZAP70 in CLL(46). The stability of epigenetic marks make them excellent molecular markers compared to measurement of levels of proteins or RNA that may change in the course of disease and therefore have to be used with caution(47). Therefore, DNA-methylation of genes whose products have been associated prognostic value (e.g. CD38) but also of novel surrogate markers like twist2 correlate with prognosis or IGVH mutation status(48).

In more recent work, FCRL (human Fc receptor-like molecules) expression was strongly associated with IGHV mutation status and FCRL2 maintained independent predictive value

by multivariate logistic analysis. FCRL2 demonstrated 94.4% concordance with IGHV mutation compared with 76.6% for CD38 and 80.4% for ZAP-70. In a study of 107 CLL patients the median treatment-free interval was 15.5 years for patients with high FCRL2 expression compared with 3.75 years for FCRL2-low patients(49).

In the gene expression studies, a number of other genes were identified with differential expression based on IGHV status, suggesting that expression levels of these genes may be used to simplify IGHV mutational assessment by use of "surrogate marker(s)". In a recent study, genes were tested with real-time quantitative polymerase chain reaction (RQ-PCR) in unpurified samples from 130 CLL patients. In multivariate logistic regression analysis expression levels of LPL, ZAP70, ADAM29 and SEPT10 were most highly correlated with IGVH mutational status. The expression of LPL was the single best predictor and in a multivariate analysis, LPL expression remained a significant predictor(50).

In a more recent analysis of 151 CLL samples (CD19+ selected) expression markers were evaluated using real-time quantitative RT-PCR (ADAM29, ATM, CLLU1, DMD, GLO1, HCSL1, KIAA0977, LPL, MGC9913, PCDH9, PEG10, SEPT10, TCF7, TCL1, TP53, VIM, ZAP70, ZNF2). Regarding individual markers, ZAP70 achieved the highest assignment rate (81%) for patients at genetic risk (IGHV unmutated, V3-21 usage, 11q- or 17p-), followed by LPL and TCF7 (76% both). This rate was improved to 88% by a 4-gene combination (ZAP70, TCF7, DMD, ATM). In multivariate analysis of treatment-free survival, IGHV mutation status and expression of ADAM29 were of independent prognostic value besides disease stage. Regarding overall survival, expression of ATM, ADAM29, TCL1, and SEPT10 provided prognostic information in addition to clinical and genetic factors(51).

It was concluded that gene expression markers are suitable for screening but not as surrogate for genetic risk factors. Many individual markers may be associated with outcome, but only few are of independent prognostic significance.

Currently there is little data to suggest that any of these markers convey predictive information with current treatment protocols. As summarized above, this may change with effective targeting of the BCR signalling components.

Genomic aberrations and gene mutations

Approximately 80% of CLL cases exhibit aberrations in a few recurrently affected chromosomal regions (Tab. 1)(8). IGHV mutated and unmutated CLL share genomic aberrations but the incidence of high risk aberrations (11q and 17p deletion) is higher in CLL with unmutated IGHV. It is important to note that the impact of genomic aberrations on outcome must be viewed in a hierarchy of the individual abnormalities. The current IWCLL guidelines deem assessment of genomic aberrations by FISH mandatory in clinical trials and desirable in general practice as pretreatment evaluation(52).

Deletion 13q14 is the structural aberration most frequently found in CLL(8). CLL with 13q14 deletion as a sole abnormality is characterized by a favorable course (Tab. 1)(8). These results are confirmed in prospective trials, but differences to genetic subgroups other than deletion 17p appear to be less pronounced with intensive first line treatment regimens. Recent work has implicated two microRNAs (miRs), miR-15a and miR-16-1, in the critical 13q14 region in CLL(53-55). While the 13q14 deletion is the most common aberration, there is currently little data to suggest that it can be used as a predictive factor. Future studies focusing on this large group of patients may investigate the de-escalation of the chemotherapy component.

Deletions of 11q22-q23. About one quarter of patients with advanced CLL exhibit 11q deletions (Tab. 1). Patients with 11q deletion have a more rapid disease progression with shorter treatment-free and overall survival times(8, 24, 27, 39). Patients with 11q deletion tend to have extensive lymphadenopathy(56). The deleted region at 11q22-23 harbours the

ATM ("Ataxia Telangiectasia Mutated") gene in almost all cases. *ATM* mutations have been shown to be present in 12% of all patients and about 1/3 of cases with 11q deletion(57, 58). The ATM protein kinase is a proximal component of the cell's response to DNA damage and DNA double-strand breaks (DSBs) as caused by chemotherapy or irradiation. ATM deficiency causes ataxia-telangiectasia, a hereditary disease characterized by neurological deficits, teleangiectasia, extreme sensitivity to irradiation, genomic instability, and predisposition to lymphoid malignancies (59). Because of the imperfect relation to *ATM* mutations, other disease-associated genes within 11q22-q23 have been investigated. Although some questions remain about the role of ATM inactivation in CLL with deletion 11q23 (and cases without deletion 11q23), efforts are underway to exploit the defects of the DNA damage machinery (see below). An approach has been suggested to bypass the impaired p53 activation upon DNA damage in ATM mutated cells by activating p53 by an alternative route to DNA damage. Small molecules that inhibit the MDM2-p53 interaction and thereby increase p53 levels have been shown to induce apoptosis in cases with deletion of ATM(60, 61), and similar approaches may be useful therapeutically.

While ATM 11q deletion has been associated with poor outcome, data from larger randomized trials suggests that primary response is independent of the presence of 11q deletion(24, 27, 39, 62, 63). Interestingly, there is evidence from clinical trials that more intensive combination chemotherapy may be particularly beneficial in patients with 11q deletion and the addition of the anti-CD20 antibody rituximab may further enhance efficacy(26, 27). In the CLL8 trial (1st line FC vs FCR), the addition of rituximab to FC increased the CR rate more than 3-fold (15.5% to 53.2%) in the subgroup of patients with 11q deletion and progression free survival ($p<.001$) as well as overall survival ($p=.004$) were markedly improved (Figure 3). These preliminary data support the concept that

chemoimmunotherapy may overcome the prognostic impact of 11q deletion and point to 11q deletion as a potential predictive factor for increased benefit by FCR treatment(64).

Trisomy 12 is a frequent aberration in CLL (10 to 20%) (Tab. 1). The oncogenes targeted by the trisomy are unknown. In prospective trials poor outcome for this group has not been confirmed with regard to overall survival when assessed according to the hierarchical model (i.e. trisomy 12 without 17p or 11q deletion) (27). The incidence of trisomy 12 does not increase with advanced stage or progression to refractory disease suggesting that this aberration is not selected for and detrimental(Tab. 1)(2, 3). Progression free survival of Binet-A patients may be relatively short, but overall survival appears to be very favourable, particularly with FC or FCR treatment (27). Cases with trisomy 12 (hierarchical) rarely show TP53 mutations – and rarely acquire these over time – a finding that may partly explain or contribute to the benign course after treatment(65).

Deletions in band 17p13 / TP53 mutations. Deletion 17p13 is found in about 7% of CLL at diagnosis and at initiation of first treatment(8, 24, 27, 39, 66). The 17p deletion virtually always affects band 17p13 including the central tumor suppressor *TP53*. Most cases with 17p deletion show loss of one copy and mutation of the remaining copy(67-69). Very few cases with 17p deletion will have a functional p53 pathway.

17p deletion has been associated with poor response to chemotherapy (alkylating agents, fludarabine based approaches) and short survival (66). Results from prospective trials confirm these results(24, 27, 39). Although rarely originating from controlled trials, there is evidence that the efficacy of “biologic” agents such as anti-CD52 antibody alemtuzumab, lenalidomide and flavopiridol is independent of the genetic background of the disease(19, 70-72) (Figure 4).

Based on this, 17p deletion may be considered a predictive marker for non-response to chemotherapy-based treatment as compared to “biologic” agents such as alemtuzumab, lenalidomide and flavopiridol. However, the demonstration of superior outcome for any treatment in CLL with 17p deletion is based on (mainly) non-randomized comparisons between trials. The current guidelines recommend testing for 17p deletion before treatment.

The first risk adapted treatment for CLL patients has thus been developed for patients with 17p deletion because of the very poor prognosis (median survival of less than 2 years from 1st treatment indication) with alkylator and nucleoside-based chemotherapy (Clb, F, FC, FCR)(24, 27, 39). Current treatment approaches in clinical trials use these agents acting independent of the p53 pathway upfront with early allogeneic stem cell transplantation(73-75).

Mutations of *TP53* are found in roughly 10% of patients with untreated CLL(66, 69, 76, 77). *TP53* mutations are associated with higher genetic complexity, although the precise impact of “complex” genetic aberrations in CLL – especially independent of *TP53* mutations – are less well defined than e.g. in acute myelogenous leukaemia(67, 69, 78, 79). Recent data suggests that the clinical behaviour of cases with only the *TP53* mutation is very similar to cases with deletion of one allele and mutation of the remaining allele(67-69).

In a recent analysis within the CLL4 trial (F vs. FC) we found *TP53* mutations in 8.2% of patients. None of the patients with *TP53* mutation showed a complete response (CR). Median progression-free survival (PFS) (23.4 vs. 61.8 months) and overall survival (OS) (29.2 vs. 84.6 months) were significantly decreased in the group with *TP53* mutation (both $p<0.001$). Interestingly, OS was significantly improved by FC treatment in the group of patients without *TP53* mutation ($p=.029$) suggesting that the absence of TP53 mutations could be considered a predictive factor for improved outcome after FC (27). If these data are confirmed, future guidelines may recommend that *TP53* mutation analysis should be incorporated into the evaluation of CLL patients before treatment initiation.

In the future we will hopefully be able to better define treatment strategies for patients with 17p deletion and TP53 mutation based on randomized trial data. Of similar importance for this subgroup of patients is the identification of drugs targeting mutant p53, which could be effective in this CLL subgroup(80).

Recurrent translocations. In contrast to other types of leukaemia or B cell lymphoma recurrent balanced translocations are rare in CLL(8, 81, 82). Translocations with breakpoints in band 14q32, where the immunoglobulin heavy-chain genes (IgH) are located, are the most common recurrent translocations in CLL. Recently, stimulation with CD40 ligand or CpG-oligodeoxynucleotides and IL-2 to increase the frequency of metaphase spreads, suggested that unbalanced translocations occurred in 34% of patients with CLL(81). In a larger series of over 500 patients, aberrations were detected in 83.0% by chromosome banding and in 78.4% cases by FISH, which only probes a limited set of regions. Recurrent reciprocal translocations were rare and mostly target known regions as the Ig-locus (in band 14q32, discussed above) or the 13q14 region with concomitant loss of genomic material(82). There is currently conflicting data on the prognostic impact of translocation targeting the IgH locus.

Novel drugs with potential to exploit biomarkers and predictive factors

Some of the most useful predictive factors used in clinical routine stem from the identification of genetic subgroups and molecular target identification as much as on the discovery of targeted therapies (e.g. PML-RAR Fusion -> retinoic acid; hormone receptor expression in breast and prostate cancer -> anti-hormone treatment; EGFR mutation and EGFR inhibition). We can predict the near future to open up many similar opportunities for treatment of patients with CLL. The BCR and downstream signal transduction pathway are obvious candidates (see above). The BCR pathway may be targetable and CLL with mutated and unmutated IGHV status are biologically and clinically distinct. While current treatment protocols in randomized

trials have not exploited this difference, this situation may soon change with substances as Syk Inhibitors or other kinase inhibitors, which exhibit early signs of differential activity based on IGHV mutation status(83, 84).

Strategies for identifying novel predictive factors in CLL

For most of the prognostic parameters discussed above, it needs to be determined whether they also have utility for predicting response to treatment in CLL. In addition to evaluating the predictive value of known prognostic factors in the context of existing therapies, it may also be possible to search for gene products that are specifically required by CLL cells harboring genetic alterations such as del(11q), del(17p), and mutant *TP53(85)*. Such an approach would enable identification of genotype-selective "vulnerabilities" that could represent novel therapeutic targets. At the same time this provides a strategy to convert established prognostic parameters into predictive factors, as patients who are candidates for molecularly targeted therapy could be selected based on the presence of the respective genetic lesions.

The concept that a cancer's sensitivity to novel, rationally designed drugs can be predicted based on an understanding of its genetic anatomy is illustrated by the recent discovery that poly(ADP-ribose) polymerase (PARP) inhibitors have efficacy in BRCA-deficient carcinomas(86). In normal cells, inactivation of PARP, which is involved in the base-excision DNA repair pathway, leads to a compensatory increase in DNA repair via homologous recombination (HR)(87), a process that requires the tumor suppressor proteins BRCA1 and BRCA2. Patients with hereditary breast or ovarian cancer should therefore be ideal candidates for treatment with PARP inhibitors as their tumor cells carry homozygous, inactivating *BRCA* mutations resulting in complete lack of HR, whereas normal tissues of these patients retain a functional copy of *BRCA1* or *BRCA2* and thus have preserved DNA repair. Accordingly,

inhibition of PARP should selectively kill BRCA-deficient cells due to their inability to counteract DNA damage and maintain genome integrity(88, 89). In support of this preclinical hypothesis, an early clinical trial showed that the PARP inhibitor olaparib has selective anti-tumor activity in cases of breast, ovarian, and prostate cancer that are associated with mutant *BRCA(86)*.

It appears likely that most cancers, if not all, are characterized by functional dependencies that are arise in consequence of specific cancer-initiating genetic alterations and represent potential targets for genotype-selective therapies. An example that may be of particular relevance to CLL is the recent observation that in TP53-deficient settings, suppression of the ATM kinase, which is involved in the cellular response to DNA damage by phosphorylating key substrates involved in DNA repair and/or cell cycle control, sensitizes tumors to DNA-damaging chemotherapy(90). Since TP53 and ATM are inactivated in a substantial proportion of CLL cases(8, 58, 91), assessment of their combined status may help predict clinical responses to established genotoxic agents such as topoisomerase II inhibitors and platinum derivatives. Furthermore, these data point to pharmacologic inhibitors of ATM as chemosensitizing agents in TP53-deficient cancer cells.

In addition to approaches that are based on knowledge of the set of genetic alterations present in a given tumor and their functional interplay, recent advances in functional genomic technologies have enabled unbiased screens for genes that are selectively required by cancer cells harboring specific genetic alterations, thereby providing a means to exploit oncogenic mutations as determinants of susceptibilities to therapies targeting these essential co-dependencies. Importantly, these approaches enable killing of cancer cells with genetic alterations that are not amenable to direct inhibition. For example, previous attempts at therapeutic targeting of oncogenic *KRAS* alleles, which occur in ~30% of human cancers,

have not met with success. A recent RNA interference (RNAi)-based "synthetic lethality" screen identified a serine/threonine protein kinase, STK33, that is selectively required by mutant *KRAS*-dependent cancer cells, irrespective of tissue origin and genetic context(92). This observation suggests that targeting of STK33 may offer a substantive therapeutic window in a broad spectrum of cancers associated with mutant *KRAS*, thus providing a rationale for the development of STK33 inhibitors(93, 94). RNAi screens have also been used to search for synthetic lethality in different, genetically defined suptypes of diffuse large B-cell lymphoma(95), and it is anticipated that similar strategies will soon be employed to study the genetic interdependencies occurring in CLL.

Summary and outlook

Many discoveries have been made in CLL biology. Based on BCR function, a subdivision is possible into two subtypes (IGHV unmutated and mutated) with distinct biological and clinical characteristics. Although the precise cellular origin of CLL remains speculative, its dependence and interaction with the microenvironment is of pivotal importance and is beginning to be utilised as a drug target. Specific aberrations (11q, 17p) and mutations (TP53, ATM) help to define distinct biological and clinical subgroups and some of these aberrations should be assessed in clinical trials as well as general practice. The biological characterisation of CLL has lead to great progress in outcome prediction and begins to impact on novel treatment strategies with the development of prognostic and predictive markers. Importantly, there is a growing number of agents acting on specific biological targets and therefore open entirely new therapeutic approaches.

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Table 1

Summary of the genetic profile of different clinical MBL / CLL cohorts.

	MBL / lymphocytosis(96)	early stage CLL(17)	Unselected(8, 68)	1st line treatment(27)	refractory CLL(19, 91)
deletion 13q14 as single aberration	39-58%	48%	36%	36%	22%
trisomy 12	18-21%	12%	15%	14%	12%
deletion 11q23	0-6%	9%	15%	21%	25%
deletion 17p13	0-3%	3%	5%	5%	31%
mutated IGHV	85-90%	61%	46%	34%	24%
unmutated IGHV	10-15%	39%	54%	66%	76%
TP53 mutation	?	?	10%	8%	37%

MBL: monoclonal B-cell lymphocytosis

*deletion 13q14 occurring as sole abnormality when assessed by FISH

Aberration	Incidence(27)	PFS (months)(27)	OS (months)(27)	Treatment arm Effect (CLL4)
no aberration	25%	70.6	not reached	FC>F (PFS)
del 13q14	50.5% (single 36%)	62.3 (single)	80,8 (single)	No difference
del 11q23	21%	43.8	73.7	FC>F
trisomy 12q13	14%	41.9	not reached	FC>F (OS)
del 17p13	5%	19.2	19.2	No difference
t 14q32	5.6%	21.8	not reached	

Tab. 2 Clinical impact of genomic aberrations in CLL

The clinical impact of these aberrations is shown by progression free and overall survival data from the German CLL4 (F vs. FC) trial of advanced CLL(27).

Practice points:

Current guidelines recommend cytogenetics (FISH) and IGHV mutation status, ZAP70 and CD38 evaluation in clinical trials.

In general practice FISH is desirable before first treatment is initiated.

While many prognostic factors have been identified in CLL, few studies identify predictive factors.

17p deletion is the strongest predictive factor for poor response with chemotherapy regimens.

TP53 mutation is likely to emerge as an important prognostic and predictive factor in CLL.

Research agenda:

Detailed analysis of biomarkers within prospective and randomized trials is an important step towards the identification of predictive factors in CLL.

The distinction of CLL with mutated and unmutated IGHV genes is borne out by distinct biological characteristics and should be amenable to targeted therapies.

CLL's sensitivity to novel, rationally designed drugs may be predicted based on an improved understanding of its genetic and immunological anatomy.

Functional dependencies that arise in consequence of specific cancer-initiating genetic alterations represent potential targets for genotype-selective therapies.

Figure legends:

Figure 1

Survival probabilities among patients in the following genetic (FISH, IGHV) categories: 17p- (17p deletion irrespective of IGHV mutation status), 11q- (11q deletion irrespective of IGHV mutation status), unmutated IGHV (homology ≥98% and no 17p or 11q deletion), and mutated IGHV (homology <98% and no 17p or 11q deletion) a) Among all 300 and b) in Binet A patients only (n=189)(44).

Figure 2

a) Kaplan-Meier analysis of overall survival (OS) stratified by serum TK(19). Black graph: TK <= 26.5 U/L, n = 45, median OS 29.0 months; red graph: TK > 26.5 U/L, n = 47, median OS 14.7 months; log rank p < .001.

b) Kaplan-Meier analysis of overall survival (OS) stratified by serum β2-MG. Black graph: β2-MG <= 4.41 mg/L, n = 57, median OS 29.4 months; red graph: β2-MG > 4.41 mg/L, n = 38, median OS 13.3 months; log rank p <.001.

Figure 3

Kaplan-Meier analysis of progression free survival of patients with 11q deletion (in the absence of 17p deletion) by treatment arm (CLL8 trial)(26). The median PFS for patients with 11q deletion receiving FC (n=58) was 27.3 months compared to 43.4 months in the FCR arm (n=80) (p<0.001).

Figure 4

a) Overall survival in cytogenetics subgroups(19). Blue graph, 17p deletion (n = 31; median, 18.3 months); gold graph, 11q deletion (n = 20; median, 22.7 months); gray graph, all other subgroups (n = 49; median, 18.6 months; log-rank P = .661).

b) Time to treatment failure in cytogenetics subgroups. Blue graph, 17p deletion (n = 31; median, 5.8 months); gold graph, 11q deletion (n = 20; median, 6.8 months; 95% CI, 3.5 to 10.9); gray graph, all other subgroups (n = 49; median, 5.4 months; log-rank P = .842).

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