CML (chronic myeloid leukemia)

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Continuous research and targeted examinations of blood and bone marrow result in various diagnostic recommendations for patients with chronic myeloid leukemia (CML).

Diagnostic recommendation

Method	Anticoagulant	Recommendation
Cytomorphology	EDTA	mandatory
Immunophenotyping	EDTA or Heparin	mandatory in (suspected) blast crisis
Chromosome analysis	Heparin	mandatory
FISH	EDTA or Heparin	optional
Molecular genetics	EDTA or Heparin	mandatory

Definition and characteristics

The incidence of CML is 1.5 per 100,000 cases per year with a median age at diagnosis of 55-60 years. Due to improved treatment options and the availability of targeted tyrosine kinase inhibitors (TKI) against *BCR-ABL1*, the survival rate and thus the prevalence of the disease has been continuously increasing.

Classification of CML

According to the WHO classification 2017, CML is a myeloproliferative neoplasm and is characterized by the presence of a *BCR-ABL1* rearrangement. Based on clinical and hematological criteria, three phases can be distinguished in CML: the chronic phase (CP-CML), the accelerated phase (AP-CML) and the blast phase ("blast crisis"; BP-CML).

CML WHO Classification 2017
(Swerdlow et al. 2017)
Myeloproliferative neoplasm

Chronic myeloid leukemia (CML), *BCR-ABL1*-positive

Table 1 compares the criteria for defining the accelerated phase and blast crisis according to WHO 2017 and ELN (European LeukemiaNet) 2013 & 2020.

Table 1: Comparison of criteria for the definition of the accelerated phase and blast crisis according to WHO 2017 (Swerdlow et al. 2017) and ELN 2013 (Baccarani et al. 2013), the latter are also valid in the updated ELN recommendation (Hochhaus et al. 2020)

	Accelerated phase		Blast phase	
	WHO 2017	ELN 2013	WHO 2017	ELN 2013
Spleen	Persistent or increasing splenomegaly, unresponsive to therapy	-	-	-
Leukocytes	Persistent or increasing high white blood cell count (>10 x10^9/l), unresponsive to therapy	-	-	-
Blast in PM or BM	10-19%	15-29% or blasts + promyelocytes ≥30% with blasts <30%	≥20%	≥30%
Basophils im PB	≥20%	≥20%	-	-
Thrombozytosis / Thrombocytopenia	>1000 x10^9/l, unresponsive to therapy <100 x 10^9/l, unrelated to therapy	<100 x 10^9/l, unrelated to therapy	-	-
Clonal chromosomal abnormalities in Ph+ cells at diagnosis	+	+	-	-
Extramedullary blast proliferation, apart from spleen	-	-	+	+

Diagnostics of CML

Cytomorphology

The cytomorphology serves to secure the diagnosis and is essential to delineate from other myeloproliferative diseases. Furthermore, it is relevant for the classification of CML into chronic phase, accelerated phase and blast crisis. In follow-up examinations under therapy, hematological remission is assessed according to cytomorphological criteria.

Immunophenotyping

Immunophenotyping is not part of the standard diagnostic program in CML at the time of initial diagnosis or in remission. However, it is important in the case of a blast phase (BP) to distinguish myeloid BP from BP of lymphatic origin.

Chromosome analysis

The translocation t(9;22)(q34;q11) is characteristic for CML
According to the WHO classification, the detection of a Philadelphia chromosome (Ph+) or a *BCR-ABL1* rearrangement is a requirement for diagnosing CML. Cytogenetically, the so-called Philadelphia chromosome is present, which usually results from a reciprocal translocation between the long arm of chromosome 9 and the long arm of chromosome 22 (t(9;22)(q34;q11)). This leads to a fusion on the molecular level between the *ABL1* (Abelson) gene located on the long arm of chromosome 9 and the *BCR* (breakpoint cluster region) gene located on the long arm of chromosome 22. This so-called standard Philadelphia translocation is found in about 90% of CML patients. The remaining patients with CML show either variant translocations (Marzocchi et al. 2011), in which one or more additional chromosomes are involved in the translocation in addition to chromosomes 9 and 22, or an unremarkable karyotype (Hagemeijer et al. 1993). In the case of an inconspicuous karyotype, a *BCR-ABL1* fusion gene is also found at the molecular level, which can be detected by fluorescence in situ hybridisation (FISH) and/or polymerase chain reaction (PCR). In these patients, the *BCR-ABL1* fusion gene develops as a result of sub-microscopic insertions of parts of the ABL1 gene into the BCR locus, more rarely of *BCR* parts into the *ABL1* locus (cytogenetically cryptic *BCR-ABL1* rearrangement). This subgroup is called Philadelphia-negative, *BCR-ABL1*-positive CML (Hagemeijer et al. 1993). With regard to clinical picture, disease progression, response to therapy and survival, both groups of patients do not differ from patients with classic, Philadelphia-positive CML according to the data available to date.

For a valid result at least 20 metaphases should be evaluated (ISCN, ELN). Chromosomal analysis also allows an assessment of cytogenetic remission.

The presence of additional abnormalities in addition to translocation 9;22 at initial diagnosis or during the course of the disease can influence the prognosis (see Prognosis) and is of therapeutic relevance (see Therapy).

The occurrence of clonal chromosomal abnormalities in Philadelphia-negative clones under TKI-therapy must be distinguished from this. The detection of such changes is of prognostic importance (see Prognosis).

FISH

FISH: rapid diagnostic validation of interphase nuclei and metaphases

The FISH analysis can confirm or exclude the presence of a *BCR-ABL1* rearrangement within 24 hours. Using FISH on interphase nuclei, 100 to 200 cells can be checked for the presence of a *BCR-ABL1* rearrangement. This technique can also be used to confirm the diagnosis in patients with Philadelphia-negative, *BCR-ABL1*-positive CML (i.e. cytogenetically cryptic *BCR-ABL1* rearrangement) - in this case a FISH analysis according to the recommendation of the ELN is obligatory (Hochhaus et al. 2020). Furthermore, FISH can be used to check the cytogenetic response to therapy on interphase nuclei as well as on metaphases. However, molecular genetic methods are much more sensitive for the determination of residual disease and are therefore obligatory for monitoring under therapy according to the ELN recommendation (Hochhaus et al. 2020).

Molecular genetics

Control of therapy response and mutation analysis in case of therapy resistance

Molecular genetics with the methods of PCR and sequencing play the central role in CML diagnostics. In addition to diagnosis (detection of the *BCR-ABL1* rearrangement), these diagnostic methods are now routinely used both to monitor the response to therapy (*BCR-ABL1* expression level) (see Table 2) and to identify resistance mutations in the event of therapy failure.

Molecular response must be calculated according to the International Scale

The *BCR-ABL1* expression level is quantified by real-time PCR. The method of RQ-PCR (quantitative real-time PCR) has the necessary high sensitivity of up to 10^{-5} (1 in 100 000). The molecular response (MR) is determined according to defined criteria (see Table 2). A good molecular response (MMR) is achieved when ≤0,1000% *BCR-ABL*^{IS} (% *BCR-ABL1/ABL1* according to International Scale) is measured.

The value of % *BCR-ABL1/ABL1* according to International Scale (IS) is calculated by multiplying the value of % *BCR-ABL1/ABL1* determined in the individual laboratory by the laboratory specific conversion factor. The laboratory-specific conversion factor is determined anew each year in comparison with a reference laboratory and enables a comparison of the % *BCR-ABL1/ABL1* values between different laboratories. The response is defined by % *BCR-ABL1/ABL1* to IS and the log reduction related to the IRIS baseline (corresponds to 100%). Furthermore, for the assignment of an MR status, the corresponding sensitivity must be achieved, which is represented by the sum of the *ABL1* copies in the performed measurements.

Table 2: Molecular response criteria (according to Cross et al. 2015), supplemented according to Hochhaus et al. 2020

Response	% *BCR-ABL1/ABL1 transcript level on the IS* (International scale)	Log reduktion based on the IRIS baseline	Minimum sum of reference gene transcripts
MR4	≤ 0,0100	4	10 000 *
MR4.5	≤ 0,0032	4,5	32 000
MR5	≤ 0,001	5	100 000

*Each value of the double measurement must be at least 10,000, otherwise no MR status may be assigned.

***BCR-ABL1* mutations**
In patients treated with TKI, there is in some cases an association between the rebound of *BCR-ABL1* expression and the emergence of TKI resistance. In this situation, sequencing for *BCR-ABL1* mutations should therefore be performed. Since certain mutations mediate resistance to individual tyrosine kinase inhibitors and other TKI may still be effective, the exact definition of the mutation is important for further therapeutic decisions (e.g. Hochhaus et al. 2020). In contrast to classical Sanger sequencing, next-generation sequencing (NGS) can also detect mutations with an allele content of 3-15%, which Soverini et al. refer to as "minor mutations" or "low-level mutations" (Soverini et al. 2013, Soverini et al. 2020). Since such subclones can expand in the course of therapy and mediate resistance to therapy, the early and sensitive detection of these mutations plays an increasingly important role in therapy decisions (Baer et al. 2016, Soverini et al. 2017 & 2020). A first prospective study underlines the clinical benefit of NGS for the sensitive detection of *BCR-ABL1* mutations in CML patients with suboptimal therapy response (Soverini et al. 2020).

***BCR-ABL1* independent gene mutations**
For a long time, CML was regarded as genetically uniform, but current molecular genetic research in this field paints a more heterogeneous picture. In CP-CML, mutations in seven genes were described as recurrent in more than one study: *ASXL1*, *DNMT3A*, *TET2*, *KMT2D*, *JAK2*, *RUNX1* and *TP53* (Branford et al. 2019). The complexity of the mutation landscape increases significantly as the disease progresses into an acute or blast phase (Branford et al. 2019, Awad et al. 2020). In this advanced stage of the disease, the most common BCR-ABL1 independent mutations are: *RUNX1* mutations (18.3%), *ICFZ1* exon deletions (16.0%), and *ASXL1* mutations (15.1%) (Branford et al. 2019). *ICFZ1* abnormalities are strongly associated with a lymphoblastic blast crisis, while *ASXL1* mutations are mainly associated with a myeloid blast crisis (Mullighan et al. 2008, Grossmann et al. 2011, Branford et al. 2018).

In many studies on the mutation landscape in CP-CML diagnosis *ASXL1* is the most frequently mutated gene (Ernst et al. 2016, Baer et al. 2017, Kim et al. 2017, Branford et al. 2018 and 2019, Nteliopoulos et al. 2019, Awad et al. 2020). In a meta-analysis of 15 different studies the incidence was 9.7% (Branford et al. 2019). The fact that *ASXL1* mutations are also found in children and young adults with CP-CML (Ernst et al. 2018) suggests that *ASXL1* mutations in the context of CML cannot be explained exclusively as an age-related phenomenon (see **CHIP in hematology**).

Some small retrospective studies indicate that the occurrence of mutations, especially in the *ASXL1* gene, correlates with therapy failure (Schnittger et al. 2014, Baer et al. 2017, Branford et al. 2018, Nteliopoulos et al. 2019, Awad et al. 2020), whereas this correlation was not found in another study (Elena et al. 2016). There is also initial evidence for a possible link between *BCR-ABL1* independent changes and disease progression (Branford et al. 2018, Nteliopoulos et al. 2019, Awad et al. 2020).

Prognosis of CML

Clinical prognosis scores
In each era of CML therapy, risk assessment scores based purely on clinical parameters have been developed; Table 3 provides an overview.

Table 3: Comparison of different clinical scores for risk stratification in CML

	Score	Sokal (1984)	Hasford (1998)	EUTOS (2011)	ELTS (2016)
	Treatment era	Busulfan/Splenectomy + intensive chemotherapy	Interferon alpha	Imatinib	Imatinib
	Prediction for	Survival	Survival	Achievement of complete cytogenetic remission within 18 months	CML caused mortality
	Risk groups	3	3	2	3
Clinical parameter	Age	x	x	-	x
	Spleen size	x	x	x	x
	Platelet count (/nl)	x	x	-	x
	Blasts in peripheral blood (%)	x	x	-	x
	Blood basophils (%)	-	x	x	-
	Blood eosinophils (%)	-	x	-	-

The EUTOS long term survival (ELTS) score described in 2016 has the greatest prognostic relevance in the current era of tyrosine kinase inhibitors (Geelen et al. 2018) and should therefore be used for risk stratification according to the ELN's recommendation (Hochhaus et al. 2020). The ELTS score differs from the Socal score, which uses the same parameters for risk classification, in the weighting of the parameters (Hochhaus et al. 2020).

The ELTS score is based on a study by **Pfirrmann et al.** that examined CML-related mortality under first-line imatinib therapy. Age, spleen size, blasts in the blood and platelet count were identified as prognostic factors for CML-related mortality. The stratification based on the specially developed ELTS score allowed the classification of the 2205 evaluable study participants into three risk groups, the majority of patients (61%) were in the low risk group, 12% were assigned to the high risk group. The probability of CML-related deaths over an 8-year period was 11% (high risk), 6% (intermediate risk), and 2% (low risk). Stratification according to the ELTS score also had prognostic relevance with regard to overall survival; the 8-year survival probability was 81% for patients in the high risk group, 84% for patients with intermediate risk and 93% in the low risk group (Pfirrmann et al. 2016).

Calculation of prognosis
Here you get to the prognosis calculation of the **ELTS score**.

Additional cytogenetic abnormalities are sometimes associated with a less favourable prognosis
In up to 10% of patients, additional cytogenetic abnormalities are observed in addition to the Philadelphia translocation at the time of initial diagnosis. The proportion of patients with additional abnormalities increases in the course of the disease and amounts to approx. 30% in the accelerated phase (Cortes et al. 2003) and 60-80% in the blast crisis (Anastasi et al. 1995, Johansson et al. 2002, Chen et al. 2017, Hehlmann et al. 2020).

Such additional chromosomal changes are a sign of disease progression. In addition to point mutations in the *ABL1* kinase domain, they also represent a possible cause of TKI resistance. Additional abnormalities can be observed even before the clinical symptoms of a blast crisis appear (Marin et al. 2008). The time interval between the occurrence of additional abnormalities and the beginning of the blast phase depends on the type of additional aberration (Gong et al. 2017). For the high-risk abnormalities described by Gong et al. (isochromosome 17q (i(17q)), aberrations of chromosome 7 (-7/del(7q)), 3q26 rearrangements, complex karyotype (≥ 2 aberrations besides t(9;22)) a rapid progress was described, so that in these patients a blast crisis occurred in the median ~2 months after the occurrence of the additional abnormalities (Gong et al. 2017).

A classification of the additional abnormalities can be made according to their frequency. A distinction is made between additional abnormalities of the so-called "major route" (trisomies of chromosomes 8 and 19, i(17q), additional Philadelphia chromosome) and the "minor route" (e.g. -7, -17, +17, +21, -Y and t(3;21)). The occurrence of "major route" additional abnormalities was associated with a worse prognosis and a higher rate of progression to the acceleration phase or blast crisis (Fabarius et al. 2011).

However, more recent data allow a classification of the individual cytogenetic additional abnormalities on the basis of their prognostic significance (Wang et al. 2016, Gong et al. 2017, Hehlmann et al. 2020). On this basis the ELN recommends a distinction between "high-risk" and "low-risk" additional abnormalities. The "high-risk" additional abnormalities are counted as "high-risk": Trisomy 8 and 19, additional Philadelphia chromosome, i(17q), -7/del(7q), aberrations of 11q23 and 3q26.2 and a complex karyotype. All other additional abnormalities belong to the "low-risk" group (Hochhaus et al. 2020). "High-risk" additional abnormalities predict a worsened response to therapy as well as an increased risk of progression (Hochhaus et al. 2020), they are therefore taken into account in therapy planning (see Therapy).

Indication of worsening of prognosis in the presence of Philadelphia negative clones
Philadelphia-negative clones can be detected in cytogenetic remission in 5-10% of patients during therapy for CML with tyrosine kinase inhibitors (Abruzzese et al. 2007, Deininger et al. 2007, Jabbour et al. 2007, Baccarani et al. 2013). The most frequent cytogenetic changes in chronic myeloid leukemia are the loss of the Y chromosome (-Y) and trisomy 8 (+8). The significance of such Philadelphia-negative clones has long been considered largely unclear (Abruzzese et al. 2007, Deininger et al. 2007, Jabbour et al. 2007, Marin et al. 2008, Groves et al. 2011). However, more recent data indicate a less favourable prognosis for these patients if the Philadelphia negative clone does not only show a loss of the Y chromosome (Issa et al. 2017).

In individual cases, an association with the development of MDS or AML has been described for the occurrence of Philadelphia negative clones - especially in the presence of monosomy 7 or the existence of cytopenia or dysplasia (Groves et al. 2011, Issa et al. 2017). The occurrence of chromosome 7 abnormalities or dysplasias was also associated with a deteriorated deep molecular response to TKI (Bidet et al. 2019).

Quality of response after 3 months predictive of deep molecular remission
The depth of the molecular response after three months is a prognostic factor for whether CML patients achieve deep molecular remission (MR4.5) after 18 months. According to ELN criteria, patients should optimally have achieved a good molecular response with *BCR-ABL1/ABL1* after IS of ≤10% after 3 months. If this is not the case, TKI resistance may be caused by one or more *BCR-ABL1* mutations and may require a change of TKI depending on the mutation status (Hochhaus et al. 2020). Achieving deep molecular remissions minimizes the risk of losing complete cytogenetic remission or MMR. However, it has not yet been conclusively clarified whether deeper molecular remission is associated with longer survival in chronic myeloid leukemia (Falchi et al. 2013, Hehlmann et al. 2014, Kantarjian & Cortes 2014).

Therapy of CML

In addition to the disease phase, therapy planning for CML also takes into account potential comorbidities as well as the possibility of a TKI discontinuation. A continuous diagnostic monitoring allows the assessment of therapy response - and therapeutic intervention in case of insufficient response, resistance, intolerance or progression. In the following, various aspects of the CML therapy algorithm are described in further detail; an overview of therapeutic decision making is also depicted in Figure 1. (Note: Click on the figure to enlarge it.)

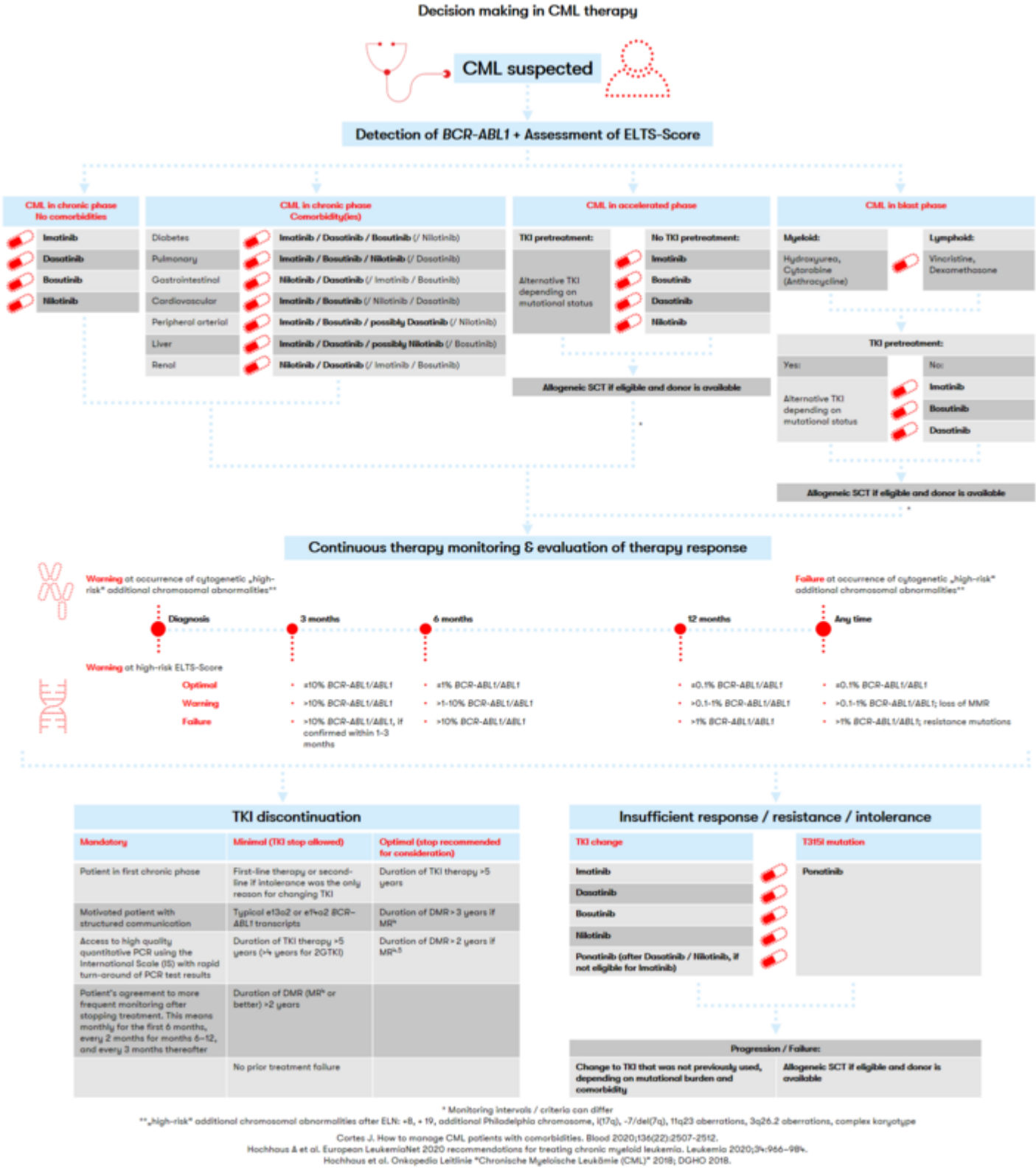

Figure 1: Decision making in CML therapy, according to Onkopedia Leitlinie CML 2018, Cortes 2020 and Hochhaus et al. 2020.

Molecular response is crucial for the course of therapy in CML

The molecular genetic analysis of the *BCR-ABL1* transcripts plays a key role in therapy monitoring. At initial diagnosis, the determination of the present transcript type is obligatory (Hochhaus et al. 2020). This is a prerequisite for molecular monitoring in the course of therapy using RQ-PCR, which is now the standard in CML diagnostics (see Table 4 and Diagnostics of CML, molecular genetics). The molecular response to TKI therapy is a decisive prognostic parameter with consequences for further therapy planning (see Table 5 and Figure 1).

Table 4: Diagnosis of CML according to European LeukemiaNet (ELN) (with modifications to Hochhaus et al. 2020)

Method	Time of investigation			
	At diagnosis	during the course	Therapy failure / resistance	(suspected) progression
Zytomorphology	x	x every 2 weeks until complete hematological remission, more often in case of hematological toxicity	-	x Determination of blast percentage
Immunophenotyping	-	-	-	Differentiation between myeloid and lymphatic blasts
Chromosome analysis (bone marrow)	x	-	x Proof / Exclusion of additional aberrations	x Proof / Exclusion of additional aberrations
FISH	(x) in Ph-negative cases	(x) for atypical *BCR-ABL1* transcript	-	-
Molecular genetics	x Qualitative PCR to detect the *BCR-ABL1* fusion and determine the transcript type; optional: RQ-PCR	x RQ-PCR every 3 months, even after reaching MMR	x Mutation analysis of the *ABL1* kinase domain	x Mutation analysis of the *ABL1* kinase domain

Definition of the molecular response to tyrosine kinase inhibitors (TKI)
The definition of optimal response to treatment for CML with a TKI or therapy failure is shown in Table 5 and Figure 1. Between optimal response and treatment failure lies a warning range in which patients require close monitoring. In the event of therapy failure, the ELN recommends that a change in therapy to an alternative TKI should be considered. A change of therapy should always be preceded by a *BCR-ABL1* mutation analysis in order to be able to make the optimal choice for an alternative TKI, depending on the type of point mutation in the *ABL1* kinase domain (Baccarani et al. 2013, Hochhaus et al. 2020). Mutation analysis is also recommended in case of repeated doubling of the *BCR-ABL1/ABL1* value (in %), suboptimal response or primary resistance.

Risk-based classification of additional chromosomal abnormalities important for therapy planning
The occurrence of "high-risk" additional chromosomal abnormalities after ELN (cf. prognosis) is considered a warning signal at the initial diagnosis and in the course of the treatment as a failure (cf. Table 5 and Figure 1). The ELN recommends treating these patients as high-risk patients, which requires close monitoring and possibly a change or intensification of therapy (Hochhaus et al. 2020) (see Table 5 and Figure 1). For "low-risk" patients, the ELN recommendations require the same procedure as for patients without additional chromosomal abnormalities.

Table 5: Definition of the response to TKI (according to Hochhaus et al. 2020)

Time	Failure	Warning (close monitoring necessary)	Optimal
Baseline		• High-risk ELTS-Score • "high-risk" ACA*	
3 months	• >10% *BCR-ABL1/ABL1* if confirmed within 1-3 months	• >10% *BCR-ABL1/ABL1*	• ≤10% *BCR-ABL1/ABL1*
6 months	• >10% *BCR-ABL1/ABL1*	• >1-10% *BCR-ABL1/ABL1*	• ≤1% *BCR-ABL1/ABL1*
12 months	• >1% *BCR-ABL1/ABL1*	• >0.1-1% *BCR-ABL1/ABL1*	• ≤0.1% *BCR-ABL1/ABL1*
Any time	• >1% BCR-ABL1/ABL1 • resistance mutations • „high-risk“ ACA*	• >0,1-1% *BCR-ABL1/ABL1* • Loss of ≤0.1% *BCR-ABL1/ABL1* (MMR)	• ≤0.1% *BCR-ABL1/ABL1*

*"high-risk" additional chromosomal abnormalities after ELN: +8, + 19, additional Philadelphia chromosome, i(17q), -7/del(7q), 11q23 aberrations, 3q26.2 aberrations, complex karyotype

Therapy-free remission
Several studies have shown that discontinuation of TKI treatment (TKI stop) is possible and safe after achieving and maintaining deep molecular remission (Campiotti et al. 2017, Narra et al. 2017, Saussele et al. 2018). Depending on the study, 40-55% of patients remained in molecular remission, although the factors predicting recurrence are still the subject of current research (Hochhaus et al. 2019). In the updated ELN recommendations, criteria for a TKI stop are given (Table 6 and Figure 1).

Table 6: Requirements for tyrosine kinase inhibitor discontinuation (according to Hochhaus et al. 2020)

Mandatory	Minimal (TKI stop allowed)	Optimal (stop recommended for consideration)
Patient in first chronic phase	First-line therapy or second-line if intolerance was the only reason for changing TKI	Duration of TKI therapy >5 years
Motivated patient with structured communication	Typical e13a2 or e14a2 *BCR–ABL1* transcripts	Duration of DMR> 3 years if MR^{4}
Access to high quality quantitative PCR using the International Scale (IS) with rapid turn-around of PCR test results	Duration of TKI therapy >5 years (>4 years for 2GTKI)	Duration of DMR> 2 years if $MR^{4.5}$
Patient's agreement to more frequent monitoring after stopping treatment. This means monthly for the first 6 months, every 2 months for months 6–12, and every 3 months thereafter	Duration of DMR (MR^{4} or better) >2 years	
	No prior treatment failure	

Recurrences after TKI stop is basically possible and usually occurs within the first 6-8 months after TKI stop (Saußele et al. 2016, Hochhaus et al. 2020). The loss of the major molecular response (MMR) is considered a recurrence (Rousselot et al. 2014). In this case, treatment should be resumed. By reinitiating therapy with the same TKI, a molecular response could be achieved again in the vast majority (90-95%) of patients (Hochhaus et al. 2020).

Overview of clinical studies of the German CML Alliance

The **linked PDF document from the University Hospital of Jena** provides an overview of currently recruiting clinical studies for adult CML patients of the **German CML Alliance**. Currently, the information is only available in German.

References

You can find the corresponding references here:
https://www.mll.com/en/diagnostic-offer/chronic-myeloid-leukemia/chronic-myeloid-leukemia-cml.html#references