VKGL vereniging klinisch genetische LABORATORIUMDIAGNOSTIEK

VKGL kwaliteitscommissie_Veldnorm

Titel: Richtlijnen verworven cytogenetica	**Doc. code: VKGL_V07**
Subspecialisme: Genoomdiagnostiek - Cytogenetica	**Versie: 04** **Ingangsdatum: 23-01-2024**

Beheerder: Eva van den Berg- de Ruiter
Centrum: UMC Groningen

Vakspecifieke toelichting op normelement 5.6 van de ISO15189-2012

Reden voor toelichting:
Als richtlijnen voor de verworven cytogenetica worden gehanteerd: European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms (Rack et al 2019a) and Guidance for reporting the interpretation of cytogenomic test results in haematological neoplasms (Rack et al 2019b).
De WHGD richtlijnen voor verworven cytogenetica zijn een aanvulling op deze recommendations en dienen derhalve in samenhang met deze documenten gelezen te worden, waarbij geldt dat de WHGD richtlijnen en aanvullende afspraken gemaakt tijdens de WHGD vergaderingen leidend zijn voor de Nederlandse praktijk. In overleg met de leden van de WHGD zijn deze richtlijnen opgesteld in het Engels.

Bereik:
Alle Nederlandse en bij de WHGD aangesloten buitenlandse laboratoria die diagnostiek verzorgen in het kader van cytogenetische analyse t.b.v. verworven chromosoomafwijkingen d.m.v. karyotypering, FISH en andere whole genome mapping technieken.

Definities: n.v.t.

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Guidelines for Cytogenomic Analysis of Acquired Disorders

Dutch addendum to the European Recommendations by Rack et al 2019

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On behalf of the "Landelijk Overleg (former Werkgroep) Hemato-oncologische GenoomDiagnostiek" (WHGD), which is a working party of the Vereniging Klinisch Genetische Laboratoriumdiagnostiek (VKGL).

The guidelines are published at the website of the VKGL (Kwaliteit > Formulieren en documenten > Veldnormen).

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General remarks

These guidelines are a supplement to and should be applied in the context of the "European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms (Rack et al 2019a) and Guidance for reporting the interpretation of cytogenomic test results in haematological neoplasms (Rack et al 2019b).
For issues not covered by the working party WHGD, European Cytogeneticists Association (ECA) guidelines, Rack et al 2019a or 2019b, other VKGL guidelines or local quality systems may apply.

Unless otherwise stated in these guidelines or working party minutes, the ECA and VGKL guidelines should be followed.

Minor changes/additions to these guidelines are documented in the minutes of working party meetings before they are integrated in a new version of the guidelines.

General recommendations

- Karyotyping and FISH are increasingly replaced by or supplemented with techniques like array, next-generation sequencing (NGS)-based techniques or optical genome mapping (OGM) (Schoumans et al 2016, Mikhail et al 2019, Neveling et al 2021, Levy et al 2024). They can be useful when insufficient metaphases are available, for the detection of acquired copy-neutral loss of heterozygosity (CN-LOH) or for cryptic aberrations which require a high resolution technique.
  Using these techniques, consideration should be taken about the capacity to detect copy number abnormalities (CNAs) and/or balanced structural variants (SVs) in the context of the reason for referral and current guidelines.
  To interpret aberrations detected with high resolution techniques in relation to classical karyotyping, in general a threshold of > 5 Mb is applied.
- For the definition of a clone see ISCN 2020 paragraph 11.1.1, page 428 (McGowan-Jordan et al 2020).
- To determine karyotypic complexity, a recommended method of counting chromosome abnormalities is published in ISCN 2020 paragraph 11.4, page 437 (McGowan-Jordan et al 2020). The definition of complexity for prognostic risk assessment is disease-specific. Relevant references are indicated in the disease-specific paragraphs in these guidelines, additional references are mentioned in ISCN 2020 paragraph 11.4.
- Thresholds and confidence limits of FISH probes should be established according to Rack et al 2019.

- When a whole genome test is performed, negative or positive results for regions specifically relevant for the reason for referral and clinical management should be reported. It is optional to report CNAs and SVs when >5Mb, irrespective of gene content, or <5 Mb when containing other known or suspected driver cancer genes. Also, CN-LOH >10 Mb extending to the telomeres are considered to be acquired abnormalities and may be reported (Schoumans et al 2016, Mikhail et al 2019).
- Amplification is defined as high copy-number gain of sequences, for which standard thresholds typically range from 3–5 fold increases over baseline ploidy (e.g., intrachromosomal amplification of chromosome 21, iAMP21 in B-ALL) to >100 copies per genome and will vary depending on the type of tumor (Mikhail et al 2019).
- Chromothripsis is defined as a copy-number profile that has alternating copy states in a single region - typically a single chromosome or chromosome arm - that contains at least ten distinct alternating copy-number segments (Mikhail et al 2019).
- Intrachromosomal complexity is defined as a summary of chromosomal regions that include more than two copy-number states, are largely confined to a single chromosome or chromosome arm, and contain at least five distinct copy-number segments. If clinically significant abnormalities (tiers 1 or 2) fall within a complex region, they may be reported individually (Mikhail et al 2019).
- Genomic complexity is defined as a pattern of chromosome instability predominantly due to structural alterations resulting in widespread gains and losses of chromosomes or chromosomal regions in the majority of chromosomes (Mikhail et al 2019).
- It should be noted that recent HUGO and WHO guidelines strongly recommend fusion genes to be written as *BCR::ABL1 (or BCR/ABL1)* and not *BCR-ABL1 (*Bruford et al 2021, WHO 2022*)*.

Sources

Sources which can be helpful with respect to the visual identification of abnormalities and association with type of haematological malignancies are:

- WHO classification of Tumours: Haematolymphoid tumours (Alaggio et al 2022, Khoury et al 2022, WHO 2022).
- International Consensus Classification (ICC) publications (Arber et al 2022, Campo et al 2022).
- Cancer Cytogenetics: Chromosomal and molecular genetic aberrations of tumor cells (Heim and Mitelman 2015).
- Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (Mitelman F, Johansson B and Mertens F). URL https://mitelmandatabase.isb-cgc.org

- Atlas of Genetics and Cytogenetics in Oncology and Haematology (Huret JL et al). URL http://atlasgeneticsoncology.org
- National protocols are published on the websites of HOVON, NVVH or SKION of Princess Maxima Center (see Appendix B).

Using these sources, one should be aware that there might be more recent publications overruling e.g. prognostic implications.

Reporting

- See publications by Rack et al 2019a and Rack et al 2019b for general guidelines on reporting.
- The interpretation and reporting of loss of the Y chromosome or trisomy 15 can be problematic. Both features are seen in bone marrow cells of elderly patients with no haematological disease, but may also occur as markers of neoplastic clones (Hanson et al 2008, Goswami et al 2015).
- In general, it should be stated whether the karyotype is male or female, unless the X and/or Y-chromosome are involved in an aberrant karyotype or no information is available about X and Y.
- In general, limitations of the test should only be mentioned when the test failed to meet the minimal requirements.
- Array based reports should always provide information on the limitations of the test, including resolution, cut off levels for detection of mosaicism and, depending on the platform, a statement that the test will not detect point mutations, balanced rearrangements, polyploidy and copy neutral loss of heterozygosity.
- Where reference is made to the International System for human Cytogenomic Nomenclature (ISCN) or World Health Organization (WHO) classification publications, the most recent versions should be used. Nomenclature for OGM is published by Moore et al 2024.
- If a patient is included in an (inter)national trial, use the risk stratification according to the trial protocol (see also minutes of the working party). If these protocols are not applicable, guidelines and recommendations from e.g. GenQA (www.genqa.org), WHO (WHO 2022) or ICC (Arber et al 2022, Campo et al 2022) should be applied.
- When relevant the report should refer to previous cytogenetic test results.

Table 1 Reporting times

Rapid test*	95% reported in 3 working days; some results should be given in <24h
Urgent referral	95% should be (preliminary) reported within 10 calendar days
Routine referral	95% should be (preliminary) reported within 28 calendar days

* When applicable, usually based on local policy

Myeloid proliferations and neoplasms

The myeloproliferative neoplasms (MPN) category in the WHO classification includes: chronic myeloid leukaemia (CML), chronic neutrophilic leukaemia (CNL), chronic eosinophilic leukaemia (CEL), polycythaemia vera (PV), essential thrombocythaemia (ET), primary myelofibrosis (PMF), juvenile myelomonocytic leukaemia (JMML), and MPN not otherwise specified (NOS) (Khoury et al 2022, WHO 2022).

In these paragraph also mastocytosis, myelodysplastic/myeloproliferative neoplasms and myeloid/lymphoid neoplasms are included. MDS and AML are described in separate paragraphs.

Chronic myeloid leukaemia

Diagnosis of CML

- The HOVON CML guideline 2023 is primarily based on the WHO classification (WHO 2022) and states that the diagnosis of CML can be based on the presence of a Philadelphia chromosome, the t(9;22)(q34;q11) and/or the *BCR*::*ABL1* fusion product by cytogenetics and/or molecular techniques. In suspected CML, FISH can be useful when no t(9;22) is detected by karyotyping and no *BCR*::*ABL1* product by molecular techniques.
- Rack et al 2019 strongly recommend that 20 cells are fully analyzed to screen for the presence of additional chromosomal abnormalities (ACAs) in Ph-positive cells.
- ACAs are classified according to the WHO 2022 classification and HOVON guidelines (WHO 2022, and table 2 in HOVON guideline 2023):
  - 3q26.2 rearrangement, monosomy 7, isochromosome 17q and complex karyotype in Ph-positive cells in CML in chronic phase are associated with an increased risk of disease progression.
  - trisomy 8,11q23 rearrangement, trisomy 19, trisomy 21 and additional Ph-chromosome in Ph-positive cells, in CML in chronic phase are <u>possibly</u> associated with an increased risk of disease progression.
- The prognosis of variant translocations (including those involving other chromosomes in addition to chromosome 9 or 22) is similar to standard t(9;22)(q34;q11) and these patients are considered equally responsive to therapy with tyrosine kinase inhibitors (TKIs).
- N.B. the term ‘accelerated phase’ is not used anymore by WHO and HOVON.

Follow-up in CML

- The WHO and HOVON guideline 2023 recommend response monitoring primarily by quantitative RT-PCR.
  Karyotyping in follow-up is only recommended in case of:
  - Persistent grade 3-4 hematological toxicity.
  - Failure of therapy to exclude progression to increased risk chronic phase or blast crisis.
  FISH can be useful for monitoring response in patients were quantitative RT-PCR is not an option.
- According to WHO 2022 en HOVON CML guideline 2023 the cytogenetic response based on the percentage of Ph+ cells is no longer relevant for follow-up.
- Clonal cytogenetic abnormalities in Ph negative (Ph-) cells occurring during treatment are mostly transient and (with the exception of -Y) some studies suggest a negative outcome. Especially monosomy 7 and del(7q) might indicate a risk of developing myelodysplasia or acute leukaemia and justify long-term follow-up bone marrow aspirates (Issa et al 2017).

Diagnosis of other MPN

- Although diagnosis of MPN is primarily based on somatic mutation analysis and cytogenetic testing is not crucial in this process, the latter may be useful to:
  - exclude (variant) translocation t(9;22)(q34;q11);
  - assess clonality;
  - assess prognosis (especially in primary myelofibrosis);
  - assess disease progression or (risk of) leukaemic transformation.
- Chapter 9 by Vandenberghe and Michaux (Heim et al 2015) gives an overview of cytogenetic aberrations (and mutations) detected in MPN at diagnosis, which may also be indicators of disease progression and leukaemic transformation.
- See also Khoury et al 2022, Arber et al 2022, Thiele et al 2023, Barbui et al 2018.

Polycythaemia vera

- The association of specific chromosomal abnormalities with prognosis in PV is summarized by Tang et al 2017 and Thiele et al 2023. They suggest to stratify into three risk groups: low-risk (normal karyotype, sole +8, +9 and other single abnormality), intermediate-risk (sole del(20q), +1q and other two abnormalities) and high-risk (complex karyotype with ≥3 abnormalities).

Essential thrombocythaemia

- Chromosome aberrations in ET seem to be associated with an inferior survival (Thiele et al 2023, Gangat et al 2022).

Primary myelofibrosis

- Cytogenetic findings are incorporated in several prognostic models for PMF (see PMF guideline www.hematologienederland.nl for the most recent recommendations on using these models. See also Tefferi et al 2021).
- The DIPSS Plus score (based on Gangat et al 2011) can be applied to all PMF patients and defines an unfavorable karyotype as: a complex karyotype or sole or two abnormalities that include +8, -7/del(7q), i(17q), -5/del(5q), del(12p), inv(3) or 11q23 rearrangements.
- The MIPSS70+ version 2.0 score (Tefferi et al 2018, Guglielmelli et al 2018) can be applied to all patients aged 70 years or younger, who are candidate for allo-stemcell transplantation (SCT). Cytogenetic abnormalities are classified according to table 3 in Tefferi et al 2018:
  - Favorable risk : normal karyotype or sole abnormalities 20q-, 13q-, +9, chromosome 1 translocation/duplication or sex chromosome abnormality (incl. –Y).
  - Unfavorable risk : all other abnormalities (including sole abnormalities +8, 7q-, translocations not involving chromosome 1, two abnormalities not including a very high risk (VHR) abnormality, single or multiple 5q-, complex karyotype without a VHR abnormality, monosomal karyotype without a VHR abnormality or sole abnormalities not otherwise specified).
  - Very high risk (VHR) : single or multiple abnormalities with -7, inv(3)/3q21, i(17q), 12p-/12p11.2, 11q-/11q23, autosomal trisomies other than +8 or +9.

Juvenile myelomonocytic leukaemia

- To diagnose JMML *KMT2A* and *BCR*::*ABL1* rearrangements should be excluded (WHO 2022, Khoury 2022). According to ICC 2022 *BCR*::*ABL1* rearrangements should be excluded (Arber et al 2022).

Mastocytosis

- In case of a suspected mastocytosis additional testing for tyrosine kinase fusions might be considered (Arber et al 2022).

Myelodysplastic / myeloproliferative neoplasms

- This category in the WHO and ICC classification includes: chronic myelomonocytic leukaemia (CMML), MDS/MPN with *SF3B1* mutation and thrombocytosis, and myelodysplastic/myeloproliferative not otherwise specified (Khoury et al 2022, WHO 2022, Arber et al 2022). In addition WHO 2022 classification includes MDS/MPN with neutrophilia and ICC 2022 includes clonal monocytosis of undetermined significance, atypical chronic myeloid leukaemia, MDS/MPN with ringsideroblasts and thrombocytosis.

Chronic myelomonocytic leukaemia

- In case of a suspected CMML rearrangements of *BCR::ABL1* or other genetic abnormalities associated with myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase fusions should be excluded (WHO 2022, Arber et al 2022).
- The association of specific chromosomal abnormalities with overall survival and risk of evolution to acute myeloid leukaemia (AML) is summarized by Itzykson et al 2018.

Myeloid / lymphoid neoplasms with eosinophilia and defining gene rearrangement / tyrosine kinase gene fusions (MLN-TK)

- In MLN-TK additional testing for *PDGFRA*, *PDGFRB*, *FGFR1*, *JAK2 (PCM1::JAK2), ETV6::ABL1* and/or *FLT3* gene fusions may be useful to detect cryptic aberrations or to confirm the involvement of these loci because these gene rearrangements may have implications for therapy choices (Khoury et al 2022, WHO 2022, Arber et al 2022).

Myelodysplastic neoplasms / myelodysplastic syndrome

Diagnosis

- According to ICC (Arber et al 2022) and the WHO classification (WHO 2022), several (cytogenetic) abnormalities in the context of persistent cytopenia are still considered to be MDS-defining. These are grouped as:
  - MDS with isolated deletion 5q (5q deletion alone, or with 1 abnormality other than monosomy 7 or 7q deletion);
  - MDS with *SF3B1* mutation (in the absence of 5q deletion, -7/del(7q), 3q26.2 aberration and complex karyotype);
  - MDS with biallelic/multi-hit *TP53* inactivation (usually with complex karyotype).
- Single or two cell loss of chromosomes 5, 7 and/or 17 poses a particular problem in karyotyping of MDS samples. To discriminate between 'random loss' and clonal abnormality, FISH or another appropriate additional test is recommended.
- Samples with hypocellular bone marrow (e.g. aplastic anaemia (AA), Fanconi anaemia) should be treated the same as MDS samples.
- Because MDS/AML is a frequent and severe occurrence in Fanconi anaemia, it is necessary to follow up patients regularly to detect transformation before the onset of an overt MDS/AML. In Fanconi anaemia patients MDS/AML can have an apparently normal karyotype but cryptic chromosomal or genomic abnormalities may be present. Therefore, analysis for gain 1q, gain 3q, -7/7q- and *RUNX1* rearrangements is required (Quentin *et al* 2011).

Interpretation, prognosis and/or risk stratification

- The Revised International Prognostic Scoring System IPSS-R (Table 2), derived from Greenberg et al 2012, Schanz et al 2012 are important standards for assessing prognosis of primary untreated adult patients with MDS.
- For calculation of the molecular international prognostic scoring system see the IPSS-M Risk Calculator (https://mds-risk-model.com) (Bernard et al 2022). Cytogenetic abnormalities important for the IPSS-M are del(5q), -7/del(7q), -17/del(17p), complex karyotype, *KMT2A*(*MLL*)-PTD, *TP53* CN-LOH and the IPSS-R score.
- If only copy number-based data are available, the report should state that additional testing for inv(3) / t(3q26) is required for evaluation of the IPSS-R.
- The IPSS is based on cytogenetics, percentage of bone marrow blasts, cytopenias, haemoglobin, platelets and absolute neutrophil count. It is recommended to describe the cytogenetic contribution to these scoring systems in the laboratory report.

Table 2 IPSS-R cytogenetic scoring system

Prognostic variable	Cytogenetic abnormality
0	-Y, del(11q)
1	Normal, del(5q), del(12p), del(20q), double including del(5q)
2	del(7q), +8, +19, i(17q), any other single or double independent clones
3	-7, inv(3)/t(3q)/del(3q)*, double including -7/del(7q), complex: 3 abnormalities
4	Complex: >3 abnormalities

***** inv(3) / t(3q) / del(3q) is interpreted as aberrations involving 3q26.2 *MECOM* (WHGD meeting 01-06-2023).

Germline predisposition

Some cases of myeloid neoplasm occur in association with inherited or *de novo* germline mutations that are characterized by specific and genetic and clinical findings, such as bone

marrow failure syndromes and the telomere biology disorders. In addition, there are disorders with predisposition to hematologic neoplasms. For a list of these disorders see table 24 in Arber et al 2022.
For recommendations for diagnosis and treatment see Raaijmakers et al 2018.

Acute myeloid leukaemia

Diagnosis

- Classification systems for acute myeloid leukaemia (AML) recognize several entities based on the presence of recurrent (cyto)genetic abnormalities (Arber et al 2022 (ICC), Döhner et al 2022 (ELN), WHO 2022).
  The relevant cytogenetic aberrations are listed in Table 3, the relevant genes (variants) are listed in Table 4. For more details and correct interpretation see Arber et al 2022 (table 25) and Döhner et al 2022 (figure 1 and table 1).
- Reporting of (preliminary) results is encouraged for early classification and risk stratification (< 1 week after sample delivery).
- Additional screening for 11q23 *KMT2A* and 3q26 *MECOM* rearrangements is highly recommended (*MECOM* rearrangements are very rare in childhood AML) as these abnormalities may be cryptic by banding analysis.
- If karyotyping has failed, additional screening with other techniques should be performed for cytogenetic markers relevant for (risk) classification.
- In case of a normal karyotype, additional screening could be performed to exclude cryptic rearrangements like *PML::RARA*, *CBFB::MYH11* or *RUNX1::RUNX1T1*, depending on the morphological subtype.
- In case of a normal karyotype in childhood AML additional screening for 11p15 *NUP98* and *CBFA2T3::GLIS2* could be considered, especially when morphology showed an acute megakaryoblastic leukaemia (Arber et al 2022, WHO 2022).

Table 3 Cytogenetic aberrations relevant for AML classification

Recurrent genetic abnormalities

t(15;17)(q24.1;q21.2) *PML::RARA*
Other recurring translocations involving *RARA*
t(8;21)(q22;q22.1) *RUNX1::RUNX1T1*
inv(16)(p13.1q22) or t(16;16)(p13.1;q22) *CBFB::MYH11*
t(9;11)(p21.3;q23.3) *MLLT3::KMT2A*
Other recurring translocations involving *KMT2A*
t(6;9)(p22.3;q34.1) *DEK::NUP214*
inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) *GATA2*, *MECOM* (*EVI1*)
Other recurring translocations involving *MECOM*
t(9;22)(q34.1;q11.2) *BCR::ABL1*
Other rare recurring translocations
t(1;3)(p36.3;q21.3) *PRDM16::RPN1*
t(1;22)(p13.3;q13.1) *RBM15::MRTFA*
t(3;5)(q25.3;q35.1) *NPM1::MLF1*
t(5;11)(q35.2;p15.4) *NUP98::NSD1*
t(7;12)(q36.3;p13.2) *ETV6::MNX1*
t(8;16)(p11.2;p13.3) *KAT6A::CREBBP*
t(10;11)(p12.3;q14.2) *PICALM::MLLT10*
t(11;12)(p15.4;p13.3) *NUP98::KMD5A*
NUP98 and other partners
t(16;21)(p11.2;q22.2) *FUS::ERG*
t(16;21)(q24.3;q22.1) *RUNX1::CBFA2T3*
inv(16)(p13.3q24.3) *CBFA2T3::GLIS2*

Table continues on page 11

Table 3 Cytogenetic aberrations relevant for AML classification (continued)

Myelodysplasia-related cytogenetic abnormalities
Complex karyotype: ≥3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities; excludes hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities.
del(5q) / t(5q) / add(5q)
-7 / del(7q)
+8
del(12p) / t(12p) / (add)(12p)
i(17q) / -17 / add(17p) / del(17p)
del(20q)
idic(X)(q13)

Table 4 Genes (mutations) relevant for AML classification

Recurrent genetic abnormalities
NPM1
CEBPA (in-frame bZIP)
FLT3-ITD
Myelodysplasia-related gene mutations
ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2

Other category
TP53

Germline predisposition
Some cases of myeloid neoplasm occur in association with inherited or *de novo* germline mutations that are characterized by specific and genetic and clinical findings, such as bone marrow failure syndromes and the telomere biology disorders. In addition, there are disorders with predisposition to hematologic neoplasms. For a list of these disorders see table 24 in Arber et al 2022.
For recommendations for diagnosis and treatment see Raaijmakers et al 2018.

Interpretation, prognosis and/or risk stratification

- For risk classification preferably the European Leukemia Network (ELN) recommendations comprising both cytogenetic and genetic abnormalities should be used, unless the patient is included in a trial for which an alternative risk classification is provided.
  Preferably the ELN 2022 risk classification is used, which is summarized in Table 5. Incidentally, for some clinical trials the ELN 2017 risk classification is still used (Table 6). For more details and correct interpretation see Döhner et al 2022 table 6) or Döhner et al 2017 table 5).
- The (risk) classification based on cytogenetic results only is a provisional one as other factors influence the final classification (gene mutations, blast percentage, prior therapy or diagnosis, germline predisposition) and risk (e.g. molecular data, minimal residual disease (MRD)). This should be mentioned in the report, especially when only normal cytogenetic results were obtained.

Table 5 2022 ELN risk classification by genetics

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1) *RUNX1::RUNX1T1*

	inv(16)(p13.1q22) or t(16;16)(p13.1;q22) *CBFB::MYH11* Mutated *NPM1*, without *FLT3*-ITD bZIP in-frame mutated *CEBPA*
Intermediate	Mutated NPM1, with *FLT3*-ITD Wild-type NPM1 with *FLT3*-ITD (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3) *MLLT3::KMT2A* Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23.3;q34.1) *DEK::NUP214* t(v;11q23.3) *KMT2A*-rearranged* t(9;22)(q34.1;q11.2) *BCR::ABL1* t(8;16)(p11.2;p13.3) *KAT6A::CREBBP* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) *GATA2*, *MECOM (EVI1)* t(3q26.2;v) *MECOM (EVI1)*-rearranged -5 or del(5q) ; -7 ; -17/abn(17p) Complex karyotype**; monosomal karyotype*** Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1,* and/or *ZRSR2* Mutated *TP53*

Footnotes for Table 5 see page 13

* Excluding *KMT2A* partial tandem duplication (*KMT2A*-PTD).

** Complex karyotype: ≥3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities; excludes hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities.

*** Monosomal karyotype: presence of two or more distinct monosomies (excluding loss of X or Y), or one single autosomal monosomy in combination with at least one structural chromosome abnormality (excluding core-binding factor AML).

Table 6 2017 ELN risk stratification by genetics

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1) *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22) *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high} Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3) *MLLT3-KMT2A* Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1) *DEK-NUP214* t(v;11q23.3) *KMT2A* rearranged t(9;22)(q34.1;q11.2) *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) *GATA2*, *MECOM* (*EVI1*) -5 or del(5q); -7; -17/abn(17p) Complex karyotype*, monosomal karyotype** Wild-type *NPM1* and *FLT3*-ITD^{high} Mutated *RUNX1* Mutated ASXL1 Mutated *TP53*

* Three or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with *BCR-ABL1*.

** Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).

- The definition of a monosomal karyotype (MK) is the presence of two or more distinct autosomal monosomies or one single autosomal monosomy in the presence of structural abnormalities, excluding marker and ring chromosomes (Breems et al 2008, Döhner et al 2022).
- Complex karyotype in AML is defined as ≥3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities, excluding hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities (Döhner et al 2022).

Precursor B or T-cell neoplasms / B or T-lymphoblastic leukaemia / lymphoma

Diagnosis

- The 2022 WHO classification recognizes several entities defined by the presence of recurrent (cyto)genetic abnormalities in B-lymphoblastic leukaemia/lymphoma (WHO 2022, Alaggio 2022, Arber 2022), the main entities are summarized in Table 7.
- When arrays are used instead of karyotyping for the detection of CNAs, in addition the relevant translocations must be investigated by FISH or alternative techniques (Rack et al 2019, Mikhail et al 2019).
- Hyperdiploid karyotypes generally show non-random trisomies and/or tetrasomies. One should be aware that they can represent a true High Hyperdiploidy (HeH: WHO definition 51-65 chromosomes; ALL Together definition 51-67 chromosomes), but also near-haploid or low hypodiploid clones which are doubled and thereby appear as pseudo-hyperdiploid. Single nucleotide polymorphism (SNP)-array can distinguish these categories based on CN-LOH for whole chromosomes. The prognosis for these two entities is different (Rack et al 2019, Creasey 2021).
- FISH for t(12;21) can also detect hyperdiploidy or intrachromosomal amplification of chromosome 21 (iAMP21), characterized by amplification of a portion of chromosome 21. iAMP21 is defined by interphase FISH with a probe for the *RUNX1* gene showing 5 or more copies of the gene per cell, with 3 or more copies on a single abnormal chromosome 21 in metaphase FISH analysis (WHO 2022).
- Additional screening for *BCR::ABL1* and *KMT2A* rearrangements is required for adult patients participating in HOVON studies. It is recommended to test for the presence of t(12;21) *ETV6::RUNX1* in young adults (< 25 year) (Rack et al 2019 table 3).
- Loci frequently involved in T-ALL are (WHO 2022):

  - T-cell receptor genes: *TRG* (7p14), *TRB* (7q34), *TRA/D* (14q11).
  - *TLX* genes: *TLX1* (10q24), *TLX3* (5q35) .
  - *NUP214::ABL1* episomes.
- In T-ALL cases, FISH for *BCR::ABL1* can be used to determine the presence of the *BCR::ABL1* fusion, but also for the detection of *ABL1* amplification (which can also be detected with a suitable *ABL1* probe). Amplification of *ABL1* is indicative for the presence of *NUP214::ABL1* episomes. The presence or absence of *ABL1* amplification should be stated in the report, as this might influence treatment decisions in T-ALL (Rack et al 2019). Deletion of *ABL1* is indicative for *SET::NUP214* fusion.
- Children aged 0-18 years should be diagnosed and classified according to the ALLTogether protocol (in the Netherlands started in July 2020). Infants 0-1 year are treated according to either ALLTogether or Interfant protocol depending on the genetic aberrations. In selected cases this protocol is also available for (young) adults.
  The ALLTogether protocol can be provided by the department of Hemato-oncology of the Princess Maxima Center.
- The ALLTogether protocol requires screening for t(9;22) *BCR::ABL1*, t(12;21) *ETV6::RUNX1*, 11q23.3 *KMT2A*, 9q34.1 *ABL1*, 1q25.2 *ABL2*, 5q32 *PDGFRB/CSF1R*, t(17;19) *TCF3::HLF*, hypodiploidy, hyperdiploidy and iAMP21. RNAseq has been validated as a robust alternative for FISH and/or RT-PCR.
- In ALLTogether a CNA profile should be determined based on copy number aberrations of *BTG1*, *CDKN2A*, *CDKN2B*, *EBF1*, *ETV6*, *IKZF1*, *PAX5*, *RB1* and the PAR1-region (*P2RY8::CRLF2*), for example investigated by array or MLPA.

Interpretation, prognosis and/or risk stratification

- For adult ALL the High Risk categories according to the HOVON treatment guideline ALL (HOVON website, version 16JUN2020, paragraph 3 table 4) are listed in Table 8.
- For patients treated according to the ALLTogether protocol the relevant genetic markers should be reported before day 15 after start treatment as they might have consequences for risk stratification.
  Results should be classified as High Risk genetics or no High Risk genetics (Table 9). The CNA profile is defined as good risk (GR) or poor risk (PR) (Table 10). Be aware that only aberrations present in an estimated frequency of ≥20% (i.e. ≥40% of blasts) that would be detected by two or more contiguous MLPA probes should be included to determine this CNA profile.
  Details are provided in the ALLTogether protocol.
- Alternatively, for prognostic implication the publications of Moorman et al 2014 or Moorman 2022 can be used or WHO 2022 (Table 7).

Acute leukaemias of mixed or ambiguous lineage

- In addition to the *BCR*::*ABL1* and *KMT2A* rearranged acute leukaemias of ambiguous or mixed lineage (ALAL/MPAL), the 2022 WHO classification recognizes two new entities with defining genetic alterations: *ZNF384* rearrangements (with fusion partners *TCF3*, *EP300*, *TAF15*, *CREBBP*) and *BCL11B* rearrangements (Khoury et al 2022, Weinberg et al 2023).

Table 7 WHO 2022 classification of precursor B-cell neoplasms

WHO 2022 entity	**Prognosis according to WHO 2022**
High hyperdiploidy (HeH) *WHO 51-65 chromosomes* *ALL Together 51-67 chromosomes*	Very favourable
Hypodiploidy *Near-haploidy 25-29 chromosomes* *low hypodiploidy 30-39 chromosomes* *High hypodiploidy 40-43 chromosomes*	Poor
iAMP21	Associated with high risk of relapse on standard therapy; overcome by more intensive therapy
BCR::ABL1 fusion	Relatively poor, improves with TKI
BCR::ABL1 like features*	Associated with high-risk clinical features
KMT2A rearrangement	Poor (might depend on translocation partner)
ETV6::RUNX1 fusion	Very favourable
TCF3::PBX1 fusion	Intermediate to relatively favourable
IGH::IL3 fusion	Uncertain (intermediate?)
TCF3::HLF fusion	Poor
Other defined genetic alterations**	

* *CRLF2* translocations (Xp22, Yp11) ; *EPOR* translocations (19p13) ; translocations involving tyrosine kinases (*ABL1* without *BCR*, *ABL2, PDGFRB, NTRK3, TYK2, CSF1R, JAK2*)
** *DUX4* (4q35), *MEF2D* (1q22), *ZNF384* (12p13.31), *NUTM1* (15q14) rearrangements, *MYC* (8q24), *PAX5* alteration *or PAX5* p.P80R (NP_057953.1)

Table 8 High risk entities according to HOVON

Philadelphia chromosome, t(9;22)(q34.1q11.2) *BCR::ABL1*
11q23 *KMT2A* (*MLL*) rearrangement
Low hypodiploidy / near-triploidy (interpreted by WHGD 12-10-2023 as masked hypodiploidy)
Complex structural and numerical chromosomal aberrations (≥5), no hyperdiploidy

Table 9 High risk entities according to ALLTogether

11q23 *KMT2A* rearrangement
iAMP21
Near haploidy (25-29 chromosomes) / low hypodiploidy (30-39 chromosomes)
t(17;19)q22;p13.3) *TCF3*::*HLF* fusion

Table 10 Definition of CNA profile according to ALLTogether

Good risk CNA profile (GR)
No deletions in *BTG1*, *CDKN2A*, *CDKN2B*, *EBF1*, *ETV6*, *IKZF1*, *PAX5*, *RB1* and the PAR1-region (*P2RY8::CRLF2*), and no amplification in *PAX5*
Deletion in/of *ETV6* solely
Deletion in/of *ETV6* plus *BTG1*
Deletion in/of *ETV6* plus *PAX5*

Deletion in/of *ETV6* plus *CDKN2A and/or CDKN2B*
Deletion in/of *PAX5* solely
Deletion in/of *BTG1* solely
Poor risk CNA profile (PR)
All other (combinations of) CNA aberrations in *BTG1*, *CDKN2A*, *CDKN2B*, *EBF1*, *ETV6*, *IKZF1*, *PAX5*, *RB1* and the PAR1-region (*P2RY8::CRLF2*)

Chronic lymphocytic leukaemia / small lymphocytic lymphoma

Diagnosis
To assign chronic lymphocytic leukaemia (CLL) patients into clinically relevant prognostic subgroups one of the following techniques is recommended on peripheral blood lymphocytes (Rack et al 2019):

- FISH with probes for 17p13.1 (*TP53*) preferably in combination with 11q22.3 (*ATM*).
- SNP-array or other genome wide tests like optical genome mapping and/or whole genome sequencing with focus on prognostic significant abnormalities that may affect clinical management (*TP53*: del(17p13.1) and/or CN-LOH for 17p) (Schoumans et al 2016; Chun et al 2018).
- Additional analysis (e.g. karyotyping, FISH for t(11;14)(q13;q32)) can be done on request or based on clinical trials.
- According to (inter)national guidelines additional *TP53* mutation analysis and *IGHV* mutation status is required (IW-CLL guidelines Hallek et al 2018). This analysis can be done in any accredited laboratory (hematology, pathology or genetics).

Interpretation, prognosis and/or risk stratification

- Results for 17p13.1 (*TP53*) must be reported (for therapy consequences) and results for 13q14 (*RB1 / MIRs / DLEU*), 11q22.3 (*ATM / BIRC3*), trisomy 12 and highly complex genome (5 or more abnormalities) are recommended to report (IW-CLL guidelines table 1 in Hallek et al 2018, Baliakas et al 2019, Leeksma et al 2021).
- For prognostication and therapy consequences see Schoumans et al 2016, Chun et al 2018, Mikhail et al 2019, Baliakas et al 2019, Leeksma et al 2021 and “Richtlijn chronisch

lymfatische leukemie".

- Highly complex karyotype/array profile with 5 or more abnormalities is an independent adverse prognosticator and related to dismal clinical outcomes, independent of clinical stage, *TP53* aberrations or *IGHV* mutation status (Baliakas et al 2019, Leeksma et al 2021).
- Patients with complex karyotype including +12, (+18),+19 displayed an exceptionally indolent profile and should not be marked as complex (Baliakas et al 2019).
- Counting complexity (following Schoumans et al 2016, Leeksma et al 2021): only CNAs ≥5Mb should be included in the determination of complexity of the array profile. The established CLL CNAs being 11q22.3 (*ATM*) and 17p13.1 (*TP53*) and 13q14 (*RB1 / DLEU*) will be called even when <5 Mb.
- Putative chromothripsis (and intrachromosomal complexity) events (where no size limit of > 5Mb is used) were counted as one event (Leeksma et al 2021, Mikhail et al 2019).

Lymphomas

Diagnosis

- To detect clinically relevant prognostic subgroups in non-Hodgkin lymphoma (NHL) or Hodgkin lymphoma (HL) karyotyping and/or FISH on fresh biopsy material or smears is recommended.
- To detect infiltration in bone marrow:
  - Karyotype 50 metaphases or,
  - Karyotype 20 metaphases and perform additional FISH if applicable.
    When FISH is performed, bone marrow smears are preferred to bone marrow cultures.
- It is highly recommended to perform FISH for translocation detection of high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* translocations (formerly double/triple hit).
- The preferred method for follow-up depends on the results of the preceding analysis.
- For HL it is recommended to perform FISH on rare giant cells, e.g. centromere 8 for detection of (near-)triploidy.
- National protocols are published on the HOVON website (see Appendix B).
- For relevant publications on diagnostics and classification of NHL and HL see Alaggio et al 2022, WHO 2022, and Campo et al 2022.

Plasma cell neoplasms and other diseases with paraproteins

Diagnosis

- Array, interphase FISH, optical genome mapping and/or whole genome sequencing should be performed on enriched plasma cells from whole bone marrow aspirates.
- The International Myeloma Working Group (IMWG) and the European Myeloma Network (EMN) recommend testing for prognostic relevant abnormalities: del(17p) (*TP53*), t(4;14)(p16;q32) (*IGH*::*FGFR3*) and t(14;16)(q32;q23) (*IGH*::*MAF*) (Sonneveld et al 2016, Caers et al 2018).
- An extended panel of targets: del(1p32) (*CDKN2C*), gain(1q) (*CKS1B*), t(4;14) (*IGH::FGFR3*), del(13q), t(14;16) (*IGH*::*MAF*), del(17p) (*TP53*), and ploidy status is recommended by the Dutch Myeloma Working Group (HOVON diagnostics guideline MM 2021).
- In patients with amyloidosis testing for t(11;14)(q13;q32) (*IGH::CCND1*) is strongly recommended (HOVON guideline amyloidosis 2020).
- Testing for 1p21-p22 (*MTF2,TMED5*), 1p12 (*FAM46C)* deletion and t(14;20)(q32;q12) (*IGH::MAFB*) is optional.
- When establishing the ploidy status by FISH, at least two chromosomes out of 3, 5, 9, 11 and 15 must be performed.
- If a normal FISH result is observed for an IGH break-apart probe, no additional FISH testing is required. In the report it must be stated that the t(4;14) and t(14;16) are not observed. In case the IGH break-apart probe shows an abnormal result, additional FISH for t(4;14) and t(14;16) is required.

Interpretation, prognosis and/or risk stratification

- For prognostic impact of cytogenetic abnormalities see HOVON diagnostics guideline MM 2021, Sonneveld et al 2016, Caers et al 2018, Sive et al 2021, Rajkumar et al 2020, Rajkumar et al 2022.
- For impact of cytogenetic abnormalities on treatment see HOVON treatment guideline MM 2021.
- The clone-size of the del(17p) must be emphasized or expressly stated in the report, because some publications are using different thresholds for prognosis (Cavo et al 2020).

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Appendix B National and international guidelines and organisations

General

https://publicatie.hematologienederland.nl/#138

https://hematologienederland.nl/kwaliteit/richtlijnen/

https://hovon.nl/en

https://www.e-c-a.eu/en/GUIDELINES.html

https://www.leukemia-net.org/home/

https://www.acgs.uk.com/quality/best-practice-guidelines/

https://tumourclassification.iarc.who.int/home

Myeloid proliferations and neoplasms

Richtlijn Chronische Myeloide Leukemie 2023, HOVON MPN werkgroep (NVVH) in preparation, final concept was available in december 2023, but not yet published on website.

http://www.leukemia-net.org/content/leukemias/cml/recommendations/index_eng.html

http://www.mipss70score.it/

Myelodysplastic neoplasms / myelodysplastic syndrome

https://hovon.nl/_asset/_public/TreatmentGuidelines/TreatmentGuidelines_Leukemia/Richtlijnen-MDS.pdf

https://ashpublications.org/blood/article/122/17/2943/31935/Diagnosis-and-treatment-of-primary-myelodysplastic (guideline ELN 2013)

https://mds-europe.org/management

Acute myeloid leukaemia

https://hovon.nl/_asset/_public/TreatmentGuidelines/TreatmentGuidelines_Leukemia/AML-richtlijn-versie-2021-06-29-definitief.pdf

Precursor B or T-cell neoplasms / B or T-lymphoblastic leukaemia / lymphoma

https://hovon.nl/_asset/_public/TreatmentGuidelines/TreatmentGuidelines_Leukemia/Richtlijn-ALL-volwassenen2020.pdf

ALL Together1 – A treatment study protocol of the ALLTogether Consortium for children and young adults (1-45 years of age) with newly diagnosed acute lymphoblastic leukaemia (ALL). Heyman et al. Most recent version available through the Princess Maxima Center, Bilthoven, the Netherlands.

Chronic lymphocytic leukaemia/ small lymphocytic lymphoma

https://hovon.nl/_asset/_public/TreatmentGuidelines/TreatmentGuidelines_Leukemia/CLL-richtlijn-final-09-06-2021.pdf

http://www.ericll.org/tp53_aberrations/#

Lymphomas

https://hovon.nl/en/treatment-guidelines/lymphoma

Plasma cell neoplasms and other diseases with paraproteins

https://hematologienederland.nl/wp-content/uploads/2022/03/Diagnostiek_myeloom_18-3-2022-DEF.pdf

https://www.amyloidose.nl/actueel/richtlijn-al-amyloidose

https://hovon.nl/en/treatment-guidelines/myeloma

Appendix C Abbreviations

AA	Aplastic anaemia
ACA	Additional chromosomal abnormalities
ACGS	Association for Clinical Genomic Science, see also ACC
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
CEL	Chronic eosinophilic leukaemia
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CNA	Copy number abnormality
CNL	Chronic neutrophilic leukaemia
CN-LOH	Copy-neutral loss of heterozygosity
DIPPS	Dynamic International Prognostic System
ECA	European Cytogeneticists Association
ELN	European Leukemia Network
EMN	European Myeloma Network
ET	Essential thrombocythaemia
FISH	Fluorescence in situ hybridisation
GenQA	Genomic Quality Assessment
HeH	High hyperdiploidy
HL	Hodgkin lymphoma
HOVON	Stichting Hemato-Oncologie voor Volwassenen Nederland
HUGO	Human Genome Organisation
ICC	International Consensus Classification
IMWG	International Myeloma Working Group
IPSS-R	Revised International Prognostic Scoring System
IPSS-M	Molecular International Prognostic Scoring System
ISCN	International System for human Cytogenomic Nomenclature
IW-CLL	International workshop on CLL
JMML	Juvenile myelomonocytic leukaemia
MDS	Myelodysplastic syndrome
MIPPS	Mutation enhanced International Prognostic System
MK	Monosomal karyotype
MLN-TK	Myeloid/lymphoid neoplasm with eosinophilia and tyrosine kinase gene fusion
MLPA	Multiplex ligation-dependent probe amplification
MM	Multiple myeloma
MPN	Myeloproliferative neoplasm
MRD	Minimal residual disease

NGS	Next-Generation Sequencing
NHL	Non-Hodgkin lymphoma
NOS	Not otherwise specified
NVVH	Nederlandse Vereniging voor Hematologie
OGM	Optical genome mapping
PMF	Primary myelofibrosis
PV	Polycythaemia vera
SCT	Stem cell transplantation
SKION	Stichting Kinderoncologie Nederland
SNP	Single nucleotide polymorphism
SV	Structural variant
TKI	Tyrosine kinase Inhibitor
VHR	Very high risk
VKGL	Vereniging Klinisch Genetische Laboratoriumdiagnostiek
WHGD	Werkgroep Hemato-oncologische Genoomdiagnostiek
WHO	World Health Organization