VIA MEDICA

REVIEW ARTICLE

Acta Haematologica Polonica 2022
Number 3, Volume 53, pages 153–165
DOI: 10.5603/AHP.a2022.0012
ISSN 0001–5814
e-ISSN 2300–7117

Chronic myeloid leukemia: where do we stand, where can we go?

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Abstract

The introduction of BCR-ABL tyrosine kinase inhibitors to the treatment of chronic myeloid leukemia (CML) has significantly changed the long term therapy results.

After an initial 12 months of therapy with tyrosine kinase inhibitor (TKI), a 3-log reduction of the BCR-ABL copies number on an international scale is possible in 22–46% of patients, depending on the TKI used. In TKI-responsive patients, long-term TKI treatment results are even better, with the BCR-ABL transcript level decreasing over time, even to the point of becoming undetectable. Therefore, an operational cure can be diagnosed in CML patients with an optimal response to 1st-line TKI treatment, a therapy duration of longer than 5–8 years, and BCR-ABL transcript level below MR4.0–MR4.5 for a period of more than two years. The latter has been the basis of multiple concepts of permanent or periodic discontinuation of TKI treatment [treatment-free remission (TFR)]. Initial TKI discontinuation clinical trials resulted in satisfactory results, with a disease recurrence rate of c.40–60% after 2–3 years. The mechanism of disease recurrence was then studied, with detailed characterization of the CML stem cells (CML SCs) immunophenotype and the mechanisms of survival and self-renewal under TKI selective pressure. A better understanding of the biology of CML allowed the formulation of new therapy concepts of CML SCs eradication, and new criteria for successful TFR qualification.

Key words: chronic myeloid leukemia, tyrosine kinase inhibitors, chronic myeloid leukemia stem cells, immune system escape, treatment-free remission, new treatment concepts

Acta Haematologica Polonica 2022; 53, 3: 153–165

Introduction

The introduction of imatinib (IM) to the treatment of chronic myeloid leukemia (CML) significantly changed the long term therapy results, with 5-year overall survival (OS) of 91.7% and progression-free survival (PFS) of 94.7% [1]. Recently, the second (2G-TKI, nilotinib, dasatinib) and third generation (3G-TKI, ponatinib) of BCR-ABL tyrosine kinase inhibitors (TKIs) have become widely used in CML patients intolerant/resistant to first line treatment with TKIs [2, 3]. Moreover, the fourth-generation allosteric BCR-ABL1 tyrosine kinase inhibitor [4G-TKI, asciminib (ABL001)] has been approved by the US FDA in CML patients resistant to first-, second- and third-generation TKIs. Its high efficacy has been proven, both in clinical trials [4] and in real-life conditions [5]. The detailed characteristics of currently used TKIs in CML patients are set out in Table I [4–31].

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Received: 03.02.2022 Accepted: 11.02.2022

Table I. Characteristics of currently used tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia patients

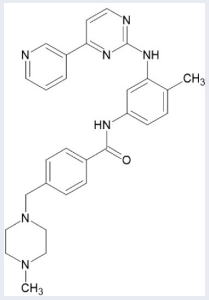
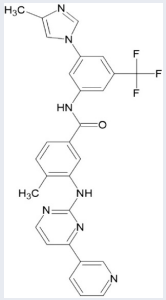
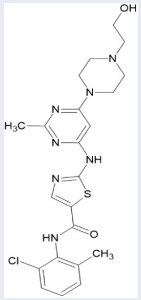
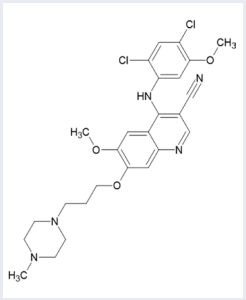
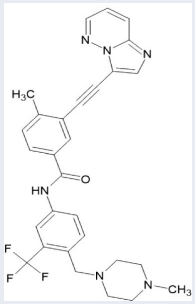
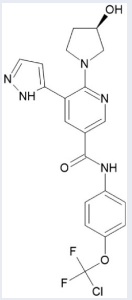
TKI/drug properties	Imatinib	Nilotinib	Dasatinib	Bosutinib	Ponatinib	Asciminib
Chemical structure						
International Union of Pure and Applied Chemistry (IUAPC) name	4-[(4-methylpiperazin-1--yl)methyl]-N-[4-methyl-3--[(4-pyridin-3-ylpyrimi-din-2-yl)amino]phenyl] benzamide	4-methyl-N-[3-(4--methyl-imidazol-1-yl)--5-(trifluoromethyl)phenyl]--3-[(4-pyridin-3-ylpyrimidin--2-yl)amino]benzamide	N-(2-chloro-6-methylphenyl)--2-[[6-[4-(2-hydroxyethyl)-piperazin-1-yl]-2-methyl--pyrimidin-4-yl]amino]-1,3--thiazole-5-carboxamide	4-(2,4-dichloro-5--methoxyanilino)-6--methoxy-7-[3-(4-methyl--piperazin-1-yl)propoxy]-quinoline-3-carbonitrile	3-(2-imidazo[1,2-b]-pyridazin-3-ylethynyl)-4--methyl-N-[4-[(4-methyl--piperazin-1-yl)methyl]-3--(trifluoromethyl)phenyl]-benzamide	N-[4-[chloro(difluoro)-methoxy]phenyl]-6--[(3R)-3-hydroxypyrroli-din-1-yl]-5-(1H-pyrazol-5--yl)pyridine-3-carboxa-mide
Molecular formula	C29H31N7O	C28H22F3N7O	C22H26ClN7O2S	C26H29Cl2N5O3	C29H27F3N6O	C20H18ClF2N5O3
Spectrum of inhibitory activity	ABL1 ARG BCR-ABL KIT PDGFR DDR1 NQO2	ABL1 ARG BCR-ABL KIT PDGFR DDR1 NQO2	ABL1, ARG, BCR-ABL, KIT, PDGFR, SRC,YES,FYN, LYN, HCK, LCK, FGR, BLK, FRK, CSK,BTK, TEC, BMX, TXK, DDR1, DDR2, ACK, ACTR2B, ACVR2, BRAF, EGFR/ERBB1-5, EPHA8,EPHB1-2, EPHB4, EPHB6, ERBB2, ERBB4,FAK, GAK, GCK, HH498/TNNI3K, ILK, LIMK1-2, MAP2K5, MAP3K1-4, MAP4K1, MAP4K5/KHS1, MAPK11/ /p38 beta, MAPK14/p38 alpha, MYT1,NLK, PTK6/ /Brk, QIK, QSK, RAF1, RET, RIPK2, SLK, STK36/ULK, SYK, TAO3, TESK2, TYK2, ZAK [6]	BCR-ABL1, ABL1, SRC, LYN, HCK	*ABL1*, *KIT, PDGFR, SRC* family, *VEGFR, EGFR, HER2, FLT3, FGFR*, and *JAK2* [7]	BCR-ABL1 [8]

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Table I (cont.). Characteristics of currently used tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia patients

TKI/drug properties	Imatinib	Nilotinib		Dasatinib	Bosutinib	Ponatinib	Asciminib
BCR-ABL tyrosine kinase inhibitory mode of action	Works by binding close to the ATP binding site, locking it in a closed or self--inhibited conformation, therefore inhibiting the enzyme activity of the protein semicompetitively [9, 10] CGP57148B, a 2-phenylaminopyrimidine derivative, has been shown to selectively inhibit the tyrosine kinase of ABL and BCR-ABL. We report here that this compound selectively suppresses the growth of colony- forming unit-granulocyte/ /macrophage (CFU-GM	Binds to and stabilizes inactive conformation of kinase domain of Abl protein [11]		Binds to ATP-binding site, but extends in opposite direction from imatinib. Binds inactive and active conformation of ABL kinase domain, requires fewer contact points with ABL, and has a greater affinity to ABL kinase domain compared to IM [12, 13]	ATP-competitive inhibitor of Src and Abl tyrosine kinases [14]	Acts as a multikinase inhibitor. Introduction of a triple bond ethynyl linker allowed spanning of bulky T315I isoleucine residue side chain in ATP-binding site, and overcame resistance to prior generation TKIs [15, 16]	Acts as an allosteric inhibitor and engages a vacant pocket at site of kinase domain normally occupied by myristoylated N-terminal of ABL1 — a motif that serves as an allosteric negative regulatory element lost on fusion of ABL1 to BCR BCR, myristoylated N-terminal is lost and ABL1 kinase is activated. By allosterically binding, to myristoyl site, asciminib mimics myristate and restores inhibition of BCR-ABL1 kinase activity [4]
BCR-ABL tyrosine kinase binding conformation	Inactive	Inactive		Active	Both	Inactive	Specifically targeting ABL myristoyl pocket
Half life time ($T_{1/2}$)	~20 hours	~17 hours		3–5 hours	32.4–41.2 hours enabling daily dose [17]	24 hours	5.5 hours (40 mg/d) 9 hours (200 mg bid)
Resistant BCR-ABL KD mutants** [8, 15, 18–23]	**Y253** **E255** **T315** M244 L248 G250	Q252 F317 M351 M355 F359 H396	**T315** L248 Y253 E255 F359	**T315** V299 F317	**T315** **V299** L248 G250 E255 F317	E250* Y253* E255* F311	**A337** **W464** **P465** **V468** **I502**

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Table I (cont.). Characteristics of currently used tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia patients

TKI/drug properties	Imatinib	Nilotinib	Dazatinib	Bosutinib	Ponatinib	Asciminib
Oral dose per day	CP 400 mg/d AP 600 mg/d BP 800 mg/d	CP 2 × 300 mg (2^{nd}-line) 2 × 400 mg (1^{st}-line)	CP 100 mg/d AP/BP 140 mg/d	CP 500 mg/d	CP 15–45 mg/d	CP 80 mg/d or 40 mg bid
Main off-target effect	Hematologic: • anemia • neutropenia • thrombocytopenia Non-hematologic: • edema (periorbital and peripheral) • muscle cramps • musculoskeletal pain • diarrhea [24]	Hematologic: • thrombocytopenia • granulocytopenia • anemia Non-hematologic: • pruritus • asthenia Cardiovascular: • cardiovascular • ischemic adverse events [25, 26]	Hematologic: • thrombocytopenia • anemia • neutropenia Non-hematologic: • endocrine disorders (gynecomastia, irregular menses, hypoglycemia, hyperglycemia, increased triglyceride and cholesterol levels) • fluid retention • nausea, vomiting, diarrhea Cardiovascular: • pericardial effusion • pulmonary artery hypertension [27, 28]	Hematologic: • thrombocytopenia • neutropenia Non-hematologic: • rash • nausea • diarrhea • vomiting • elevated serum aminotransferases [29]	Hematologic: • anemia • thrombocytopenia • neutropenia Non-hematologic: • rash • elevated serum lipase • pancreatitis Cardiovascular: • hypertension • chest pain [30]	Hematologic: • thrombocytopenia and/or neutropenia Non-hematologic: • hepatic impairment • asymptomatic amylase and/or lipase elevations Cardiovascular: • hypertension • pericardial effusion [4, 5, 31]

*Increase in IC50 for ponatinib as a sole anomaly typically not leading to clinical resistance, which is observed in cases of a compound mutation including *T315I*; **strong resistance is indicated in bold; CP — chronic phase; AP — acceleration phase; BP — blastic phase; bid (bid in die) — twice daily

Current results of CML treatment

Data originating from clinical trials and from real life studies has confirmed the high efficacy of TKIs in CML patients in the chronic phase in terms of the 3-log reduction of BCR-ABL1 copies number (major molecular remission, MMR) with a well standardized real-time PCR technique in the blood after 12 months of treatment. MMR response rates differed depending on the type and dose of TKI used, and amounted to 22–36.9% for IM 400 mg once a day, 44% for nilotinib 300 mg twice a day, 43% for nilotinib 400 mg twice a day, 46% for dasatinib 100mg once a day, and 47.2% for bosutinib 500 mg once a day [1, 32–34].

The long-term TKI treatment results show that in the TKI-responsive patients the BCR-ABL transcript level continuously decreases over time, to the point of becoming undetectable. The overall cumulative incidence of the confirmed MR4.5, and stable MR4.5 (4.5log reduction in BCR-ABL1 copies number in international scale, IS) after eight years of IM therapy is 51.7% and 36.5%, respectively [35]. Real life data is in agreement with the computer simulation results, showing treatment time to MR4.5 to be 10.7 and 9.1 years in IM-treated patients participating in the IRIS trial (training set) and the CML IV trial (validation set), respectively [36]. This data forms the basis of the concept of an operational cure and the permanent or temporary discontinuation of TKI treatment [treatment-free remission (TFR)] [37].

Data concerning the frequency of deep molecular responses (DMR, defined as the reduction of the transcript level below MR4 or MR4.5) on TKI treatment has been accumulated subsequently. Its analysis allowed the formulation of minimal criteria which should be fulfilled for a TFR attempt in CML patients, including a low or intermediate Sokal score, a typical BCR-ABL1 transcript type at diagnosis, a chronic phase of CML in the past history, an optimal response to 1st-line TKI treatment, a TKI therapy duration of longer than eight years, a DMR at the time of qualification, and a duration of DMR monitored in a standardized laboratory of longer than two years. Initial study results showed that only 10–12% of patients on IM appeared to be eligible for the discontinuation of a TKI [37, 38]. Subsequent data has shown that only a minority of CML patients reaching the sustained DMR on TKI therapy were candidates for the discontinuation of treatment without the risk of a molecular disease recurrence. Until now, many possible solutions have been proposed for optimizing the process of CML patient qualification for a TFR attempt. Initially, only those patients with an MR5.0 or an undetectable BCR-ABL transcript were qualified for the TFR studies (STIM pilot, STIM1, STRIM2, ASTIM and TWISTER) [39–42]. In all the aforementioned trials, a molecular CML recurrence was defined as BCR-ABL1 positivity in two consecutive assessments, or the loss of a MMR (or ≥1 log increase of the transcript level in STIM1 and STIM 2 trails). The TFR rate after the median follow-up of 12 months was 61% (STIM2), 50% after 18 months (STIM pilot), 61% after 31 months (A-STIM), 47% after 42 months (TWISTER), and 38% after 77 months (STIM2). The EURO-SKI trial results confirmed that there was no difference in the TFR rates between patients with >MR4.5 and MR4 [43, 44]. Afterwards, patients with a stable MR4.0 were also enrolled in TFR clinical trials. Unfortunately, to date there is no consensus regarding the criteria which should be used for the qualification of CML patients for an TFR attempt to minimize the probability of MMR loss. According to the different criteria for TFR proposed by Hughes et al., Rea et al., Hochhaus et al., and Radich et al., the probability of CML patient recruitment for TFR varies from 9.5% to 55% [45–49]. Molecular recurrence-free survival after TKI cessation, according to the eligibility criteria proposed by Hughes et al., Rea et al., Radich et al., and Hochhaus et al., varies from 35% to 60% after a follow-up of 45–100 months [45–49]. Therefore, considerable efforts have been made to further optimize the criteria for TFR attempt qualification. The incorporation of genomic data into future model(s) of unfavorable risk assessment will likely change the currently used algorithms for TFR qualification [50–53].

Nowadays, the NCCN and ELN guidelines recommend the re-initiation of TKI therapy at the time of molecular recurrence, defined as a loss of MMR after the first TFR attempt [48, 54].

The 2G-TKI discontinuation studies results are limited. They include 327 patients with 1st-line TKI failure (IM, interferon/IM), and 190 patients treated upfront with nilotinib. Unfortunately, different criteria were applied in different studies for the TFR attempt qualification before the 2G-TKI discontinuation (MR4–MR4.5), and different definitions of molecular disease relapse (loss of MMR to loss of MR4.5) were applied. For these reasons, the interpretation of TFR rates at 24 months (ranging from 44 to 62.8 months) is difficult [50, 55–59].

According to recent data, the re-initiation of TKI therapy results in secondary molecular remission in a significant proportion of CML patients. Therefore, the idea of a second TFR attempt has been tested. In 2017, Legros et al. presented data concerning 70 patients successfully treated with IM who attempted a first TFR (IM = 60, nilotinib = 5, dasatinib = 5) and, after disease relapse, underwent a second TFR attempt. The TFR probability at 6, 12, 24, and 36 months after the second attempt to discontinue TKI was established at 66%, 48%, 42%, and 35%, respectively [60]. However, it should be mentioned that a second TFR is not yet considered standard practice.

It was previously documented that the majority of patients in DMR still harbor leukemic cells [61] capable of initiating disease relapse upon the withdrawal of TKI treatment [41]. The presence of CML leukemic stem cells in IM-treated patients in DMR with undetectable levels of mRNA was

confirmed in 32.8% of samples by Pagani et al. with the help of a genomic DNA Q-PCR assay [62]. The mechanism of the persistence of CML SCs in TKI-responsive CML patients in DMR is not fully understood. Recently, it was postulated that CML SCs are protected from MHC class I-dependent CD8+ cytotoxic T lymphocytes (CTLs) elimination in the bone marrow by regulatory T cells (Tregs) expressing tumor factor 4 receptor (Tnfrsf4). This hypothesis is based on the observation that Tregs are preferentially localized in the CML bone marrow close to CD8+ CTLs, and that TNFRSF4 mRNA levels correlate with the expression of Treg-restricted transcription factor FOXP3 [63]. Moreover, it has been shown that human CML stem cells are insensitive to IM, despite the inhibition of BCR-ABL activity [64]. This observation forms the basis of a hypothesis that primitive CML cells are not oncogene-addicted [65, 66].

CML, TKI treatment and immune system function

It has been postulated that TKI-induced changes in immune system functioning may affect the risk of a CML relapse after TKI discontinuation (Table II) in a different way. In CML patients in the chronic phase, suppression of the immune system is present at the time of diagnosis. This is mainly caused by the promotion of the expansion of myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg) by cytokines/chemokines released by proliferating CML progenitor cells. MDSC originate from malignant *BCR-ABL1* clone mediate immunosuppressive activity via a number of mechanisms, including the increased production of reactive oxygen, nitrogen species, arginase-1 (molecule inhibiting T cells), and TGF-β1 [67].

Moreover, MDSC can recruit Treg and inhibit cytotoxic T cells [68]. The immune escape of malignant cells is also promoted due to increased expression of the programmed death-1 (PD-1) inhibitory molecule on the CD4+/CD8+ T cells [69] and PD-L1 upregulation on CML cells [70, 71]. Quantitative and functional defects of the innate effector natural killer (NK) cells and the cytotoxic T-lymphocyte responses to leukemia-associated antigens (CTL-LAA) broaden the spectrum of immune system defects in CML patients at the time of diagnosis [69].

The significance of the immune system for a successful TFR was confirmed by the Immunostim study, which documented an association between elevated peripheral blood natural killer (NK) cells and a positive clinical outcome following IM discontinuation [85]. Other immunomodulatory effects of TKI administration leading to immune system re-activation and restoration of effector-mediated immune surveillance were recently documented. TKI treatment resulted in the restoration of NK cell receptor repertoire and an enhanced NK cell function, a decrease of immune suppressors (MDSC, Treg and T lymphocytes PD-1+), the restoration of LLA-CTL responses including PD-1 downregulation to normal levels, and an increase in DC number and antigen presenting cell function [67]. The restoration of immune system function in CML patients on TKI seems to be optimal

Table II. Off-target effect of BCR-ABL tyrosine kinase inhibitor's administration on immune system in patients with chronic myeloid leukemia

Off target effect on immune system	Imatinib	Nilotinib	Dasatinib	Bosutinib	Ponatinib
Hypogammaglobulinemia (via inhibition of Burton kinase) [72–74]	+				
Decrease in memory-B-cell count (via inhibition of Burton kinase) [72, 75]	+				+
B-cell immune responses impairment [74, 76]	+	+	+		
Treg and effector T cells function impairment (via inhibition of Src kinase) [77–79]	+	–	+	+	
Decreased cytotoxicity and reactivity of NK cells [79]	+	–	+		
Abrogation NK cells cytokine production [79]			+		
Decreased proliferation and function of CD8+ T lymphocytes [80]		+			
LGLs expansion [81, 82]	–	–	+		
Increased proliferation of cytotoxic (CD3+CD8+) T cells and/or NK cells (CD3–CD16+/56+) [83]			+		
Decrease of NK cells count [75]	+			+	+
Decrease of MDSCs level [84]	?	?	?	?	?

NK — natural killer cells; Treg — regulatory T lymphocytes; LGLs — large granular lymphocytes; MDSCs — myeloid-derived suppressor cells

after reaching MR4.5 (BCR-ABL1 ≤0.0032%), a time when the increased effector NK cell number and function and T cell immune responses, and reduced numbers of PD-1+ CD4+/CD8+ T cells and monocytic MDSC are maximal [69].

Another question concerns the role of immune surveillance by natural killer and T cells in maintaining a successful TFR and disease activity control [66]. In 2017, Jacomet et al. postulated that the deficiency of iNKT/InnateCD8+ T cells axis is present in CML patients [86]. Their hypothesis was confirmed by the observation of CML patients in TFR ≥2 years carried out by Cayssials et al. documenting the increase of functionally active innate CD8(+) T-cells [NK-like KIR/NKG2A(+)] and their number [87]. The presence of specific CTLs directed against CXorf48 (cancer testis antigen) expressed in LSC is also correlated with the relapse rate in CML patients who discontinued imatinib after maintaining complete molecular remission for more than two years [88]. The success of the TFR attempt likely depends on the 'quite normal' efficiency of the immune system. This is possible only if MR4.5 response to TKI treatment is reached, and when NK cells number and effector T-cell cytolytic function is increased, and when PD-1 expression on the T-cell and numbers of monocytic MDSCs is reduced [69].

Chronic myeloid leukemia stem cells

CML SCs are not fully defined yet in terms of immune and functional characteristics [89]. CML SCs likely share an immunophenotypic profile with normal hematopoietic stem cells (HSCs) and reside in the CD34+/CD38–/Lyn– cell fraction [90]. Also, CD25 and CD44 are expressed in both CML SCs and healthy HSCs [91, 92]. On the other hand, interleukin-1-receptor accessory protein (IL-1RAP) and CD26 [dipeptidyl peptidase-4 (DPP4)] are aberrantly expressed on the CML SCs' surface, but not in normal CD34+ cells [93–95]. CML SCs can self-renew and generate large numbers of leukemic progenitor cells (CD34+CD38+) with the capacity to differentiate or enter a dormant state. In 2012, BCR-ABL1-independent CML SCs was postulated by Hamilton et al. [96]. They documented that the process of survival and self-renewal of CML LSCs was associated with activation of the cellular signalling process, including cell-intrinsic and cell-extrinsic survival pathways.

The first group includes abnormal signaling via the Janus kinase–signal transducer and activator of transcription 3/5 (JAK–STAT3/5), WNT/β-catenin, sonic Hedgehog (Hh) or PIK3/AKT pathways, abnormal function of protein phosphatase-2 (PP2A), promyelocytic leukemia (PML) protein, dual specificity tyrosine phosphorylation regulated kinase 2 (DYRK2), repression of autophagy process, deregulated expression of microRNAs (i.e. the upregulation of miR-29a--3p, miR660-5p, has-mir183), disturbed epigenetic regulation of genes expression by enhancer of Zeste Homolog 2 [a member of the polycomb repressive complex 2 (PRC2)], deregulation of fatty acid cellular metabolism due to arachidonate 5-lipoxygenase (ALOX5)-associated abnormalities of arachidonic acid conversion to leukotrienes required for malignant cells self-renewal, and BCR-ABL1-related autocrine production of cytokines resulting in growth factors-independent STAT5 activation [i.e. interleukin (IL) 3, granulocyte colony-stimulating factor (G-CSF)]. Other key regulators influencing the apoptosis, self-renewal, cell fate and senescence process of CML SCs include abnormal transforming growth factor-β (TGF-β)/Forkhead box O (FOXO) interaction and Musashi 2 (Msi2)/Numb of NOTCH signaling [97–105].

Bone marrow microenvironment and CML SCs

CML SCs reside in the same bone marrow microenvironment (BMM) as normal stem cells. The cross-talk between CML SCs and BMM is mediated by soluble factors like cytokines [IL-3, IL-1α/β, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, and interferon gamma (IFN-γ), myostatin propeptide (MSTNpp), sCD14, IL-21 and IL-13v, and CCL-28], chemokines [i.e. C-X-C motif chemokine ligand 12 (CXCL12)] secreted by mesenchymal stromal cells, and osteoblastic cells), growth factors via autocrine and/or paracrine mechanisms, and cell-to-cell (mesenchymal stromal cells, osteoblastic cells endothelial cells, neurons) direct interactions via surface adhesive molecules (i.e. β_1-integrin). The aforementioned interaction may have resulted in an enhanced proliferation, quiescence, and drug resistance of CML SCs [106–109]. Lastly, the role of miR-126 secreted by the endothelial cells in the process of CML SC quiescence and self-renewal control has been postulated [110]. Similarly, the role of miR-300, expressing dual anti-proliferative and PP2A-acivating properties, in the process of CML SCs quiescence and persistence has recently also been confirmed [111].

Due to the high diversity of CML SCs (subclonal character, different metabolic characteristics, and molecular and immunophenotypic fingerprints) and high inter- and intra-patient heterogeneity, the possibility of a common, unified strategy for CML SC eradication has been neglected. What is more, CML SCs eradication is now irrelevant due to the significant improvement of TKI long term treatment results and encouraging results of TFR attempts [112].

The current concept of chronic myeloid leukemia treatment with tyrosine kinase inhibitors based on the reduction of measurable residual disease and the recovery of immune system function is set out in Figure 1.

New therapeutic approaches concerning CML treatment

A better understanding of the biology of CML has allowed the formulation of a number of new therapy concepts for

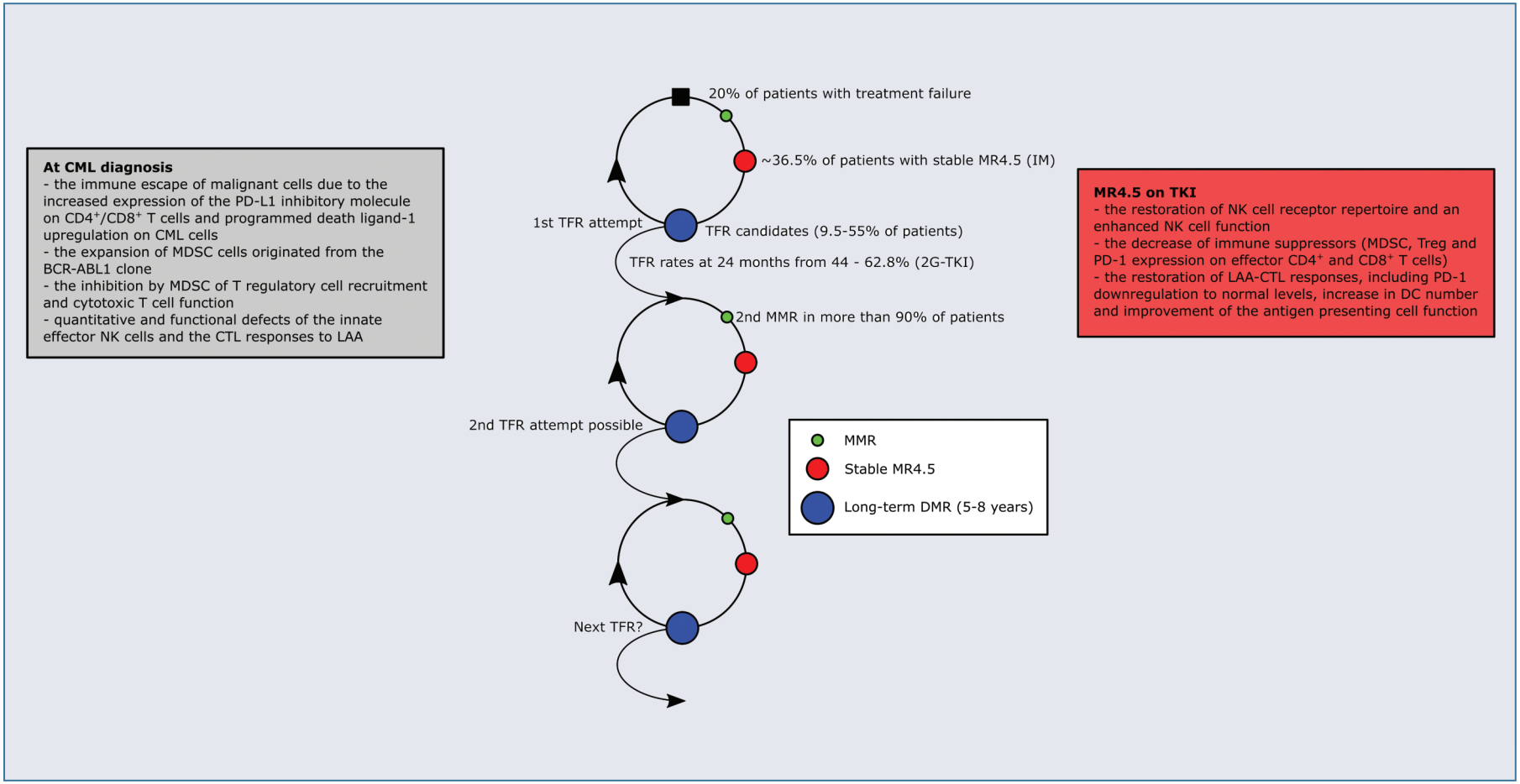

Figure 1. Current concept of chronic myeloid leukemia (CML) treatment with tyrosine kinase inhibitors based on reduction of measurable residual disease and immune effector recovery; CTL — cytotoxic T lymphocytes; LAA — leukemia-associated antigens; MDSCs — myeloid derived suppressor cells; MR — molecular response in log scale; PD-L1 — programmed death-ligand 1

the eradication of leukemic cells with the help of immunotherapy or chimeric antigen receptor–engineered T cells (CAR-T) directed against CXorf48 (cancer testis antigen) or IL1RAP (IL-1 receptor accessory protein) [88, 113]. Recently, quiescent primitive SCs insensitive to IM subpopulation of CML were identified in a CD36+ cell subpopulation with the help of an RNA-seq study [114]. This data forms the basis of the concept of antibody-based therapeutic targeting of CML SCs [112, 114, 115].

Moreover, an innovative strategy based on a liposome loaded with the BCL2 inhibitor venetoclax exploiting begelomab (an anti-CD26 antibody) has been proposed to target positive CML SCs CD26+ [116] more selectively.

In our opinion, its use, in combination with TKI and other drugs targeting alternative CML LCs survival pathways, should be the future of combinatorial therapy for the eradication of CML stem cells.

CML and the future

Integrative genomic analysis reveals cancer-associated mutations at the diagnosis of CML in patients. WES, RNA-seq and gene expression profiling studies have identified a number of molecular aberrations in addition to *BCR-ABL1*, among others affecting epigenetic regulators such as *ASXL1*, *DNMT3A*, *TET2*, *SETD1B* and transcription factors (*IKZF1*, *RUNX1*) [51–53, 117]. Its presence could also potentially influence the process of making therapeutic decisions in future [53, 118].

Conflict of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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