Tyrosine kinase inhibitors for the treatment of acute myeloid leukemia: Delineation of anti-leukemic mechanisms of action

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ARTICLE INFO

Article history:
Received 16 March 2011
Accepted 12 May 2011
Available online 1 June 2011

Keywords:
Apoptosis
Differentiation
Epidermal growth factor receptor
Tyrosine kinases
Erlotinib
Lapatinib

ABSTRACT

Initially, tyrosine kinase inhibitors (TKIs) were developed as targeted therapies that would solely interfere with aberrant tyrosine kinase activation in malignant cells. Nevertheless, preclinical and clinical studies demonstrated that TKI also exhibit “off-target” effects, that is effects not mediated by the assumed mechanisms of action. We and others showed that the epidermal growth factor receptor (EGFR) inhibitors erlotinib and gefitinib exert potent antineoplastic effects on EGFR-negative myeloblasts from patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Here, we undertook a side-by-side comparison of the anti-leukemic efficacy of four different TKI in MDS and AML. Besides the EGFR inhibitor erlotinib, which served as a point of reference, we employed the dual EGFR/HER2 TKI lapatinib, as well as the multikinase inhibitors dasatinib and sorafenib. All four drugs had anti-leukemic effects on cell line models of MDS/AML in vitro as well as on malignant blasts from MDS/AML patients ex vivo. We explored the biological phenomena underlying this anti-leukemic efficacy. Since it is established that a therapeutic benefit in MDS/AML can be conveyed by induction of cell cycle arrest, apoptosis and/or differentiation, we deciphered the individual contribution of these three phenomena to the anti-leukemic action of each of the four TKI. The concomitant assessment of the panel of TKI enables us thus to define (and quantify) their differential capacity to impact on the three biological phenomena, and provide further evidence that these mechanisms are not solely explained by on-target effects.

1. Introduction

Acute myeloid leukemia (AML) are heterogeneous diseases with regards of morphology, cytogenetics, molecular biology and prognosis [1–6]. Recent years have seen the advent of targeted therapies aiming to exploit the presence of molecular alterations that are restricted to the malignant cells. Thus, sorafenib was developed to suppress the oncogenic tyrosine kinase activity arising from mutations in the FMS-like tyrosine kinase 3 (FLT3) gene on chromosome 13q12, an alteration detected in up to 35% of AML patients [7]. Likewise, dasatinib was designed as a dual SRC/ABL-kinase inhibitor for the treatment of chronic myeloid leukemia, where aberrant ABL-kinase activity represents the crucial pathogenic event [8]. A recent study provided evidence that dasatinib is also able to inhibit the kinase activity of mutant FLT3 and c-KIT [9].

The concept of targeted therapies assumes that the mode of action of small molecules is – due to their specificity – confined to the neoplastic cell population, thus avoiding the unwarranted side effects seen upon conventional treatment (such as chemotherapy...
with cytotoxic agents). Obviously, truly targeted therapies should not exhibit any beneficial effects in the absence of their presumed target.

However, many preclinical and clinical studies have provided evidence that tyrosine kinase inhibitors (TKIs) exhibit off-target effects and that these effects cannot be explained by the TKI’s impact on their presumed targets. Consistent with these observations, we and others demonstrated previously that two small molecule TKIs, erlotinib and gefitinib, originally designed to inhibit the receptor tyrosine kinase epidermal growth factor receptor (EGFR), may exert therapeutic off-target effects on MDS and AML cells (which do not express the EGFR) [10–14]. Whereas erlotinib and gefitinib are considered “pure” EGFR-1-inhibitors since they inhibit EGFR-1 but none among the three other receptors of the EGFR family, lapatinib was designed as a dual inhibitor of EGFR-1 and HER2, another member of the EGFR-family. Lapatinib has been FDA-approved for certain types of breast cancer, since both receptors are frequently overexpressed in this malignancy, in particular in cancers with aggressive clinical behavior and poor clinical outcome [15].

Based on these considerations, we here assessed the anti-leukemic efficacy of the four different TKIs, including erlotinib (serving as a point of reference, since we previously described its capacity to induce apoptosis, cell cycle arrest and differentiation in myeloid cells [12]), lapatinib, dasatinib, and sorafenib, in a side-by-side comparison in MDS- and AML-derived cell lines and primary myeloblasts.

2. Patients, materials and methods

2.1. Patient samples

Patient samples were assessed after obtaining informed consent according to the Declaration of Helsinki. The diagnosis of AML and MDS was determined by morphological examination of peripheral blood and bone marrow, according to the WHO and FAB classification, and cytogenetic analysis was performed according to conventional methods.

2.2. CD34+ cell selection, cell lines and culture conditions

Peripheral blood or bone marrow mononuclear cells (MNCs) were isolated using a ficoll-paque plus density gradient (Amersham Biosciences, Sunnyvale, CA, USA). To obtain CD34+ cells from MNC, a positive selection with the MiniMacs system (Miltenyi Biotec, Bergisch Gladbach, Germany) was carried out. Subsequently, CD34+ cells were cultured in Iscove modified Dulbecco medium (IMDM, Gibco, Carlsbad, CA, USA) supplemented with 1 % L-glutamine (Gibco), 100 units/ml penicillin–streptomycin (Gibco), and BIT 9500 serum substitute (200 μg/ml transferrin, 10 μg/ml insulin, 2 % bovine serum albumin, purchased from StemCell Technologies, Grenoble, France) at a final concentration of 1 × 10^5 cells/ml and a combination of cytokines consisting of interleukin-3 (10 ng/ml), interleukin-6 (10 ng/ml), thrombopoietin (50 ng/ml) (all purchased from Peprotech, Neuvilly-sur-Seine, France), FLTR3-Ligand (100 ng/ml) and stem cell factor (50 ng/ml) both obtained from Miltenyi Biotec. MOLM-13 and HL-60 cells were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). All cell lines were cultured in FCS-supplemented RPMI 1640 (Gibco). Unless otherwise specified, cells were seeded at a concentration between 1.0 and 1.5 × 10^5 cells/ml.

2.3. Drug preparation and storage

Erlotinib and sorafenib were purchased from LC Laboratories (Woburn, MA, USA), and lapatinib and dasatinib were obtained from Selleck Chemicals (Houston, TX, USA). Stock solutions of all TKI were prepared by dissolving compounds in 0.01 % DMSO and stored at –20 °C. ATRA (all trans retinoic acid, Sigma-Aldrich, St Louis, MO, USA) was also dissolved in 0.01 % DMSO and stock solutions stored at –20 °C.

2.4. Assessment of cytotoxicity

MTT assays were carried out according to the manufacturer’s instruction (CellTiter 96, Promega, Madison, WI, USA). In detail, individual wells of 96-wells microculture plates were seeded with 100 μl of the respective cell suspension containing 50 × 10^5 cells/ml and the indicated concentrations of TKI. After 24, 48 and 72 h of incubation at 37 °C, 20 μl of the tetrazolium (MTS, Promega) and phenazine ethosulfate (PES, Promega) containing reagent were added and the cells once more incubated for 1 h at 37 °C. Subsequently, 25 μl of 10 % SDS was added to stop the reaction, and absorbance quantified at 490 nm in a Fluostare Optima reader (BMG, Champigny-sur-Marne, France). Absorbance of RPMI supplemented with 10 % FCS served as a reference.

2.5. Measurement of cell cycle distribution

To determine cell cycle distribution, 5 × 10^5 cells were collected for each condition and washed once with ice-cold PBS. Cells were permeabilized with 100 μl of 0.5 % Triton (Sigma-Aldrich). 100 μl of RNase (20 μg/ml) (Invitrogen, Carlsbad, CA, USA) was added 15 min before staining with 50 μg/ml propidium iodide solution (Sigma-Aldrich) and samples incubated for 1 h at +4 °C. Analyses were performed using Cellquest® (BD Biosciences, San Jose, CA, USA) software.

2.6. Quantitation of apoptosis

As described previously [12,16–22], cells were stained with the vital dye propidium iodide (PI; 1 μg/ml; Sigma, Steinheim, Germany) and DiOC6(3) (3,3 dihexyloxocarbocyanine iodide; 20 nM; Molecular Probes, Eugene, OR, USA) for 15 min at 37 °C to determine loss of the mitochondrial transmembrane potential. Apoptotic cells were quantified by cytofluorometric analysis using a FACSCan (Becton Dickinson, Mountain View, CA).

2.7. Evaluation of differentiation

Morphological assessment of differentiation was carried out after May–Gruenwald–Giemsa (MGG) staining of cytopsin, as described previously [12]. Briefly, slides were fixed in methanol (Sigma–Aldrich) for 15 min, stained for 5 min in May–Grunewald solution (Sigma–Aldrich), and for 10 min in Giemsa solution (Oxoid, Dardilly, France), rinsed (buffer at a pH 6.8) and air-dried. Signs of differentiation (decrease of cytoplasmic basophilia and nuclear/cytoplasmic ratio, appearance of granularity, lobulation of the nucleus) were assessed in at least 100 cells/condition.

To assess differentiation by cytofluorometry, cells were harvested, washed and stained with an APC-conjugated anti-CD11b antibody (clone D12, Becton Dickinson), as described previously [12]. To block unspecific binding to human Fc-receptor expressing cells, samples were pre-incubated with FcR blocking reagent (Miltenyi Biotec). Analysis was carried out on live cells, which were gated based on forward and side scatter patterns. Isotypic mouse IgG2a-APC (Becton Dickinson) was used to determine threshold parameters.

NBT reducing activity was determined as described by Kohroki et al. [23]. Briefly, after the incubation of cells with the drugs, cells were harvested, mixed with freshly prepared Hanks buffer (PAA Laboratories GmbH, Pasching, Austria) containing 1 μg/ml phor-
bol-12-myristate-13-acetate (Sigma–Aldrich), and 2 mg/ml nitroblue tetrazolium chloride (NBT) (Sigma–Aldrich) and incubated for 30 min at 37 °C. The degree of NBT reducing activity was determined in at least 100 cells/condition using a Zeiss microscope (Thornwood, NY, USA). DMSO- and ATRA-incubated cells served as negative and positive controls of differentiation, respectively.

3. Results and discussion

3.1. Impact of the TKI erlotinib, lapatinib, dasatinib, and sorafenib on the viability of MDS/AML cell lines

MOLM-13 (established from a patient with acute myelomonocytic leukemia after initial MDS) and HL-60 (established from a patient with primary AML) cells were cultured in the absence or presence of equimolar doses of the four distinct TKI for 24 h, 48 h and 72 h, followed by the determination of cell loss by the tetrazolium reduction assay. All four TKI decreased the viability of the two myeloid cell lines in a dose- and time-dependent manner. Nevertheless, the anti-leukemic efficacy of the four TKI varied considerably in both cell lines (Fig. 1A and B). Whereas low doses of erlotinib and sorafenib consistently decreased the viability of MOLM-13 and HL-60 cells over time, this effect was not observed upon treatment with similar doses of lapatinib and dasatinib. Rather 1 μM of lapatinib and dasatinib increased the metabolic activity of HL-60 cells (48 h), an effect that was also observed in MOLM-13 cells incubated with lapatinib (but not with dasatinib). Nevertheless, in the high-dose range (that is at 15 μM) the anti-leukemic efficacy of lapatinib was comparable to that of equimolar doses of sorafenib leading at 72 h of incubation to the near-to-complete loss of metabolically active myeloid cells (Fig. 1).

This first series of experiments demonstrated that all four TKI share the capacity – although to a different extent – to decrease the viability of myeloid cell lines.

![Fig. 1. Impact of the TKI erlotinib, lapatinib, dasatinib, and sorafenib on the viability of MDS/AML cells. MOLM-13 (A) and HL-60 (B) cells were incubated with the indicated doses (given in μM below the x-axis) of the 4 TKI, and cellular viability was assessed by MTT assay after 24, 48 and 72 h of incubation. Changes in viability are given as percentage of cells as compared to non-treated control samples. This experiment was repeated at least three times, yielding comparable results. Graphs show representative results of one experiment carried out in triplicates (mean ± standard deviation).](image-url)
3.2. Anti-proliferative potential of the 4 TKI in MDS/AML cell lines

In order to discern the exact biological phenomena responsible for the decrease in cell viability, we first tested the hypothesis that the different TKI might block the cell cycle progression of myeloid cells. We therefore recapitulated the aforementioned incubation schedule in MOLM-13 and HL-60 cells and assessed the drugs’ impact on cell cycle progression by cytofluorometric analysis. As described previously [12], erlotinib induced a dose-dependent cell cycle arrest in the G1 phase. As shown in Fig. 2, both dasatinib and sorafenib, but not the dual inhibitor lapatinib, also increased the percentage of cells in the G1-phase of the cell cycle. Of note, sorafenib’s capacity to block cell cycle progression in MOLM-13 cells became already detectable at low doses (1 μM) and thus surpassed those of the other tested TKIs (Fig. 2A and B). These anti-proliferative effects were already detectable at 24 h and were maintained at 48 h of incubation (Fig. 2 and data not shown). With increasing drug doses, the appearance of hypodiploid cells (that is cells in the sub-G1-phase of the cycle) was observed, notably upon treatment with lapatinib and sorafenib (Fig. 2A–C).

Altogether, these results establish that the decrease of viability observed in the MTT assay is at least in part mediated by a drug-induced arrest in cell cycle progression. Moreover, the increase of hypodiploid cells (most pronounced under incubation with sorafenib) already observed after 24 h of incubation suggests that TKI might also induce apoptosis.

3.3. Pro-apoptotic effects of the four TKI on MDS/AML cells

To corroborate the hypothesis that the observed decrease of viability was caused – at least in part – by the induction of apoptosis, experiments were repeated followed by cytofluorometric quantitation of cells stained with the vital dye propidium iodine (PI) and the mitochondrial transmembrane potential (∆Ψ_m)-sensitive probe DiOC_3(3) (which measures the ∆Ψ_m, whose dissipation is indicative of incipient cell death) [17–21]. As described in previous studies, at early time points (i.e. at 24 h and 48 h) erlotinib had no or little apoptosis-inducing potential [12]. In contrast, the dual EGFR/HER2-inhibitor lapatinib exhibited a considerable apoptosis-induction capacity, an effect observed already at 24 h of incubation and increasing over time. At high dose levels, this degree of apoptosis-induction was comparable to that observed under equimolar doses of sorafenib, and largely exceeded those of dasatinib (and erlotinib) (Fig. 3). These results show that both lapatinib and sorafenib exert their anti-leukemic efficacy to a considerable extent by inducing “early” apoptosis (that is already detectable at 24 h of incubation), and moreover demonstrate that the capacity of these drugs to induce apoptosis (at least at 24 h and 48 h) largely exceeds the degree of cell death observed with dasatinib and erlotinib.

3.4. Differentiation-inducing capacity of the four TKI in MDS/AML cells

To complete the characterization of the anti-leukemic effects of the 4 TKI, we compared their ability to overcome the AML-associated differentiation block. In contrast to the aforementioned experiments, we did not compare equimolar doses of the 4 TKI, and rather preferred to use selected doses that were compatible with prolonged cell survival, namely for six days at which the parameters of differentiation were evaluated. Experiments were carried out with ATRA, an established inducer of differentiation in AML blasts [24], serving as positive control. Since TKI had been dissolved in DMSO (which can stimulate differentiation [25]), parameters of differentiation were evaluated with respect to control cells incubated with equimolar doses of DMSO. In a first series of experiments, we assessed four signs of morphological differentiation, i.e. TKI-induced reduction of cytoplasmic basophilia, decrease in the nucleo-cytoplasmic ratio, appearance of nuclear lobulation and finally cytoplasmic granulation. May-Grunenwald-Giemsa staining of MOLM-13 cells (Fig. 4A and B) demonstrated that dasatinib induced a dose-dependent decrease in cytoplasmic basophilia and in the nucleo-cytoplasmic ratio. Dasatinib also led to the appearance of dented nuclei and cytoplasmic granulation. Sorafenib decreased cytoplasmic basophilia to the same extent as dasatinib, an effect observed together with the appearance of prominent cytoplasmic vacuoles. As reported previously, MOLM-13 cells were unable to differentiate in response to erlotinib [26]. Likewise, lapatinib had no or little impact on cellular differentiation (Fig. 4A and C). In HL-60 cells, erlotinib (10 μM) and sorafenib (1 μM) both decreased basophilia of the cytoplasm and the nucleo-cytoplasmic ratio to a similar extent, whereas dasatinib (5 μM) once more exhibited the highest differentiation-inducing capacity (Fig. 4C). Thus dasatinib diminished basophilia of the cytoplasm concomitantly with the nucleo-cytoplasmic ratio, and induced the appearance of nuclear lobulation and cytoplasmic granules. Next, we determined the degree of CD11b expression, a marker indicating myeloid differentiation, in TKI-treated MOLM-13 (Fig. 4C and D). Approximately 15% of MOLM-13 cells exhibited a certain degree of CD11b expression upon DMSO-incubation, and this expression only slightly increased with 1 μM of erlotinib. The failure of higher doses of erlotinib to induce CD11b expression in MOLM-13 cells might be explained by the time of assessment (6 days of incubation) at which erlotinib-sensitive cells might have already succumbed to apoptosis. Whereas 1 μM of lapatinib did not increase CD11b surface expression (in accordance with the lack of differentiation observed in the MGG stainings), a higher dose (2.5 μM) slightly increased CD11b intensity (Fig. 4D). Once more, dasatinib exhibited the most pronounced capacity to increase CD11b surface expression (Fig. 4C and D). In contrast, the decrease in cytoplasmic basophilia observed in sorafenib-treated MOLM-13 cells was not accompanied by the cell surface exposure of the myeloid marker CD11b (Fig. 4A and D).

To corroborate the TKI-driven differentiation, we quantified the enzymatic activity of alkaline phosphatase by means of the NBT-reduction assay after an incubation period of 6 days in MOLM-13 cells. Once again, the NBT-reductive activity was highest in cells treated with dasatinib, which was as active as the positive control, ATRA (Fig. 4E and F).

In conclusion, we demonstrate that within the panel of the four evaluated TKI, dasatinib had the most pronounced ability to overcome the AML-associated differentiation block. Noteworthy, at the concentration of dasatinib at which HL-60 and MOLM-13 cells underwent differentiation (5 μM), they did not enter the apoptotic program (compare Figs. 3 and 4).

3.5. Comparison of the anti-leukemic efficacy of the four TKI in primary MDS/AML cells

To finally evaluate the anti-leukemic efficacy of the four TKI in malignant blasts, bone marrow MNC from 8 AML or MDS patients were selected for CD34⁺-positivity and incubated with the indicated doses of erlotinib, lapatinib, dasatinib and sorafenib (Fig. 5). At 48 h of incubation, the frequency of dead (PI⁺) and dying (DiOC₃(3)low) cells was determined by cytofluorimetry. As could be expected from the results obtained with cell lines, the response of individual patient samples to the four different TKI was highly heterogeneous. As previously reported [12,26], erlotinib’s ability to induce apoptosis at 48 h of incubation was limited, but nevertheless tended to be higher – at least in the samples assessed here – than cell death induced by dasatinib (with the caveat that we did
Fig. 2. Anti-proliferative potential of the 4 TKI in MDS/AML cells. Impact of the four TKI applied at the indicated doses (given in μM below the x-axis) on cell cycle progression of MOLM-13 and HL-60 cells. Cell cycle distribution was determined by cytofluorometry following PI staining at 24 h of the incubation period. (A) Depicts representative FACS stainings of MOLM-13 cells at 24 h. (B) Shows quantitation in MOLM-13 cells. (C) in HL-60 cells at 24 h (mean ± standard deviation). Data are representative for three independent experiments.
Fig. 3. Pro-apoptotic effects of the four TKI on MDS/AML cell lines. The myeloid cell lines MOLM-13 and HL-60 were treated with the indicated doses (given in μM below the x-axis of the graphs) of the 4 TKI and stained with DiOC₆(3)/PI assessing dissipation of the mitochondrial transmembrane potential and loss of viability. Representative FACS diagrams of MOLM-13 cells after 48 h of incubation (A), as well as quantitation of apoptosis in MOLM-13 (B) and HL-60 (C) cells at 24 h and 48 h of incubation (mean ± standard deviation). This experiment was repeated at least three times, yielding comparable results.
Fig. 4. Capacity of the TKI to overcome the AML-typical differentiation blockade. The myeloid cell lines MOLM-13 and HL-60 were incubated for 6 days with 0.01% DMSO (serving as a negative solvent control), 1 µM of ATRA (serving as a positive control), as well as with the indicated doses of the four TKI. (A) Representative May–Gruenwald–Giemsa staining of MOLM-13 cells, (B) quantitation of the percentage of MOLM-13 cells exhibiting at least two morphological signs of differentiation (that is a decrease in cytoplasmic basophilia, a reduction of the nucleo-cytoplasmic ratio, appearance of nuclear lobulation and/or cytoplasmic granules). Percentages were evaluated by examining at least 100 cells/condition; (C) representative FACS overlays of MOLM-13 cells depicting TKI-induced CD11b expression (black line) as compared to the isotype (shaded grey); (D) quantitation of TKI-induced CD11b-expression in MOLM-13 cells; (E) representative slides depicting morphology/staining of MOLM-13 cells assessed in the NBT-reduction assay; (F) respective quantitative assessment demonstrating the NBT-reducing capacity under the different drugs; (G) representative May–Gruenwald–Giemsa staining of HL-60 cells.
Noteworthy, in the tested patient cells, the apoptosis-inducing capacity of 5 μM lapatinib was comparable to that of 5 μM of sorafenib (Fig. 5A and B), a drug already evaluated in clinical trials of AML [27]. Although the number of patient samples was too small to draw any substantial conclusion – notably with respect to TKI-sensitivity and hematological characteristics such as karyotype – the observed apoptosis sensitivity in patient samples (i.e. the higher cellular mortality with sorafenib and lapatinib compared to erlotinib and dasatinib) resembled the pattern exhibited by the myeloid cell lines (compare Figs. 3 and 5).

3.6. Concluding remarks

Although many studies have evaluated the anti-neoplastic effects of the four TKI inhibitors tested here, to our knowledge
these drugs have not been compared for their antineoplastic effect by direct side-by-side analysis. In addition, important insights originate from this assessment of the four TKI’s impact on three potential therapeutic mechanisms, namely blockade of cell cycle progression, induction of cell death and terminal differentiation. Collectively, our results corroborate the notion that the anti-leukemic effects of TKI are not restricted to cells expressing the respective target. The quintessential example of a therapeutically useful off-target effect is given by imatinib, which was initially designed to “specifically” inhibit aberrant BCR-ABL activation, specific of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia with the Philadelphia chromosome. However, imatinib was subsequently shown to inhibit the deregulated activity of c-KIT and the platelet-derived-growth-factor-receptor (PDGFR) [28]. Subsequently, TKIs antagonizing a broader spectrum of targets were developed taking into account the insight that the majority of malignancies exhibits a multitude of deregulated pathways, and that treatments that are “too specific” may have limited impact on these heterogeneous diseases. Thus, dasatinib, which belongs to the generation of multi-kinase TKI, was FDA-approved for the treatment of CML patients resistant or intolerant to imatinib [29]. Besides targeting the abl-kinase, dasatinib also inhibits c-KIT, PDGFR, and various members of the Src-kinase family [29]. Noteworthy, these effects are not restricted to malignant cells, possibly explaining — at least in part — the immunomodulatory action of this agent [29]. Of note is the here demonstrated increase in cell viability observed under low doses of dasatinib in HL-60 cells, in line with results of Han et al. [30] who found that low doses of dasatinib could induce an “stimulatory effect on cell proliferation” in long-term cultures of ex vivo AML cells. Our results also corroborate pre-clinical and clinical evidence for dasatinib’s capacity to overcome the AML-typical differentiation block [31,32]. Previous reports demonstrated that dasatinib enhances ATRA-induced differentiation in AML cells and that single agent therapy with this TKI may even induce major in vivo differentiation of AML blasts resistant to conventional chemotherapeutic treatment [31,32]. Like dasatinib, sorafenib targets many molecules and related pathways (including c-KIT, vascular endothelial growth factor receptor, PDGFR, as well as Raf-MEK-ERK signaling), and is of interest in AML for its capacity to antagonize deregulated signaling induced by the internal tandem duplication (ITD) of FLT3, associated with an exceptionally poor prognosis under standard chemotherapeutic treatment [7]. We found that sorafenib’s anti-leukemic efficacy was more pronounced in MOLM-13 than in HL-60 cells, knowing that MOLM-13 cells were established from a 20-year-old patient with AML associated with the FLT3/ITD mutation [33]. In addition we found the anti-leukemic efficacy of sorafenib to reside in its ability to inhibit cell cycle progression and to induce apoptosis. As detailed above, we and others showed that the EGFR-inhibitors erlotinib and gefitinib exhibit “off-target” effects in EGFR-negative myeloid blasts [10–14,17]. Noteworthy, clinical evidence from three case reports led strong support to the notion that erlotinib is beneficial in some high-risk MDS and AML patients [11–13] and two prospective clinical studies, including one from our group, are currently assessing the tolerance and anti-leukemic activity of erlotinib in those disorders (NCT00977548, NCT01085838). Since lapatinib shares the target of EGFR with erlotinib, we included that drug in the panel of tested TKIs, hypothesizing that it might exert comparable effects. As observed for dasatinib in HL-60 cells, low doses of lapatinib temporarily increased cell numbers in both myeloid cell lines, in agreement with the absence of any cell-cycle-arresting effect at the early time points of assessment. In contrast, higher concentrations of lapatinib induced a considerable degree of apoptosis in HL-60 and MOLM-13 cells, as well as in patient-derived blast cells.

In conclusion, we performed a systematic and exhaustive comparison of four different TKI and their biological effects on representative myeloid cell lines as well as on ex vivo blast cells from AML and high-risk MDS. We thus demonstrated that all four TKI exhibit anti-leukemic efficacy and delineated the individual contributions of cell cycle arrest, apoptosis and differentiation to their anti-neoplastic action.

Acknowledgements

EL receives a scholarship from Post Accueil INSERM, ST from INCA, MS from Fondation de Recherche médicale, MT from the Ligue contre le Cancer. GK is supported by Cancéropôle Ile-de-France, Institut National du Cancer, Fondation de France, Association Laurette Fugain, Cent pour Sang la Vie, Agence Nationale de la Recherche, and the European Commission (Active p53, ApoSys, ChemoRes. Death-Trans, RIGHT, Trans-Death). SB is supported by Fondation de France and Cent pour Sang la Vie.

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