Hodgkin-Reed/Sternberg (HRS) cells of classical Hodgkin lymphoma show aberrant expression and activation of several receptor tyrosine kinases (RTK) in the majority of cases. Therefore, we tested whether tyrosine kinase inhibitors (TKI) already in clinical use or late stages of clinical trials have antiproliferative effects on HRS cell lines and evaluated the targets, affected signaling pathways, and mechanisms of cell death and resistance. Sorafenib and lestaurtinib had antiproliferative effects on HRS cell lines at concentrations achievable in patients. Sorafenib inhibited platelet-derived growth factor receptor (PDGFR) α, TRKA and RON, caused decreases in total and phosphorylated amounts of several signaling molecules, and provoked caspase-3-independent cell death, most likely due to endoplasmic reticulum stress as indicated by upregulation of GADD34 and GADD153 and phosphorylation of PERK. Lestaurtinib inhibited TRKA, PDGFRα, RON, and JAK2 and had only a cytostatic effect. Besides deactivation, lestaurtinib also caused activation of signaling pathways. It caused increases in CD30L and TRAIL expression, and CD30L/CD30 signaling likely led to the observed concomitant activation of extracellular signal-regulated kinase 1/2 and the alternative NF-κB pathway. These data disclose the possible use of sorafenib for the treatment of Hodgkin lymphoma and highlight NF-κB activation as a potential novel mechanism of resistance toward TKIs. Mol Cancer Ther; 12(2); 173–83. © 2012 AACR.
on activated signaling pathways, and the modes of cell death induction and resistance of both TKIs.

Materials and Methods

Cell lines

HRS cell lines (HDLM-2, KM-H2, L-428, L-1236, and U-H01) were obtained from the DSMZ and cultured as recommended. Cell line identity was determined by the DSMZ by STR DNA fingerprinting and ensured by continuous culture for less than 6 months. Hematopoietic neoplasm U-2932, SU-DHL-8, SU-DHL-10, KARPAS-422, SU-DHL-1, KARPAS-299, DG-75, RAMOS, DAUDI, RAJI, JURKAT, L-363, K-562 and HL-60, carcinoma MCF7, MDA-MB-468, A-549, HTB-56, HELA, and fibroblast NIH-3T3 cell lines were obtained from several colleagues (R. Küppers, Essen, Germany; C. Müller-Tidow, Münster, Germany; A. Neumann, Münster, Germany). As they were used only for comparisons of the antiproliferative effects of sorafenib and lestaurtinib, they were not authenticated by the authors. Numbers of viable cells were determined with a CASY TT cell counter as technical duplicates (Innovatis AG).

MTT assay for metabolic activity

Cells were plated at 1 × 10^5 cells/mL (L-428 and KM-H2) or 2 × 10^5 cells/mL (L-1236) and incubated with the indicated drug or solvent alone. To quantify enzymatic reductase activity corresponding to metabolic activity, the In Vitro Toxicology Assay (Sigma-Aldrich) was used, and the concentration of the dye converted from MTT was measured in duplicate at 570 nm with a NanoDrop (Peqlab).

Apoptosis analysis by flow cytometry

For analysis of cell death induction after treatment with lestaurtinib or sorafenib, L-428, L-1236, and KM-H2 cells were stained with FITC-AnnexinV (BD Biosciences Pharmingen) and propidium iodide (PI; AppliChem) according to the manufacturer’s instructions and analyzed by flow cytometry.

Immunoprecipitation of RTKs

Cells were lysed in NP-40–based lysis buffer (Pierce Co-Immunoprecipitation Kit, Thermo Scientific) supplemented with 2 mmol/L Na_3VO_4 and Complete Mini Protease Inhibitor Cocktail (Roche Applied Science). Cell lysates (250 μg protein) were incubated with 1 μg of anti-Ron (C-20) or anti-Trk (C-14) antibodies (Santa Cruz Biotechnology), and immune complexes were collected by binding to Dynabeads Sheep anti-Rabbit IgG (Life Technologies). Proteins were eluted by boiling Dynabeads in SDS sample buffer.

Western blot analysis

Western blot analysis was conducted as described in Supplementary Materials and methods with primary antibodies specified in Supplementary Table S1. Results shown here are representative for at least 2 independent experiments. Densitometric analysis was conducted with the open access image processing program ImageJ.

Reverse transcription PCR and quantitative real-time PCR

Total RNA was extracted with the RNeasy Plus Micro Kit (Qiagen). The cDNA was synthesized using the 1st Strand cDNA Synthesis Kit (Roche Applied Science). For quantitative PCR analysis, the Applied Biosystems Power SYBR Green Master Mix System (Life Technologies) was used. Technical duplicates were conducted for each sample. Primer sequences are given in Supplementary Table S2. Identity of all PCR products was verified by sequencing.

Affymetrix GeneChip Human 1.0 ST Array analysis

KM-H2 was incubated for 24 hours with 1 μmol/L lestaurtinib or dimethyl sulfoxide (DMSO), and total RNA was extracted and analyzed with GeneChip Human 1.0ST arrays. Processing of the microarrays and initial bioinformatic analyses were conducted by the Integrated Functional Genomics Unit of the Interdisciplinary Center for Clinical Research at the Medical Faculty of the University of Münster (Münster, Germany). After scaling data were imported into GeneSpringGX (Agilent Technologies) and normalized using the default normalization methods. Mean values of replicate gene arrays were used to determine changes in mRNA expression by pairwise comparison (P < 0.05) of lestaurtinib-treated samples to control samples. To identify gene ontology (GO) categories, differentially expressed genes (fold change > 2) were analyzed with GeneCodis (16). All data about the microarray analysis have been deposited at ArrayExpress under accession number E-MEXP-3565.

Results

Sorafenib and lestaurtinib inhibit proliferation of HRS cell lines

Screening of 9 TKIs revealed that only sorafenib and lestaurtinib reduced proliferation of 3 of 5 HRS cell lines after 48 hours in a concentration-dependent manner and at concentrations achievable in patients (IC_{50} for sorafenib: 8 μmol/L for L-1236 and L-428; 5 μmol/L for KM-H2; IC_{50} for lestaurtinib: 10 mmol/L for L-1236; 300 mmol/L for L-428 and KM-H2; Fig. 1A–E and Supplementary Tables S3 and S4; refs. 17, 18). At longer incubation times also, HRS cell lines HDLM-2 and U-H01 showed sensitivities comparable with other HRS cell lines (Fig. 1F and G), most likely due to their longer doubling times. These sensitivities were in line with 2 recently published studies for HRS cell lines (14, 15). Incubation of other lymphoma, leukemia and carcinoma cell lines with 5 μmol/L sorafenib, a concentration at which other cell lines without RTK mutations showed sensitivity (19–21), and 300 mmol/L lestaurtinib, a concentration at which cell lines with FLT3 or JAK2 mutations were sensitive (22, 23), revealed that sensitivities toward the multikinase inhibitor sorafenib were comparable for all cell lines (Fig. 1F) and that HRS cell
lines were less sensitive toward lestaurtinib than all other tested cell lines (Fig. 1G).

Cell death induction by sorafenib but not lestaurtinib in HRS cell lines

As sorafenib and lestaurtinib inhibited growth of HRS cell lines, we determined whether this was due to a cytostatic effect or cell death. Therefore, we incubated L-428, L-1236, and KM-H2 cells with the TKIs and used Western blotting (WB) for active, cleaved forms of the effector caspase-3 and flow cytometric analysis after Annexin V/PI staining to detect apoptosis (Fig. 2).

Sorafenib treatment lead to a substantial decrease of viability in 3 HRS cell lines as detected by Annexin V/PI staining but not by Western blotting for activation of the effector caspase-3 in L-428 and KM-H2. Lestaurtinib induced apoptosis in L-1236 as detected by Annexin V/PI staining and caspase-3 Western blotting but had, in contradiction to previously reported findings (15), only a cytostatic effect on L-428 and KM-H2 cells as no substantial apoptosis induction could be detected by either means.

Targets of sorafenib and lestaurtinib in HRS cell lines

To identify the targets of sorafenib and lestaurtinib in HRS cell lines, we analyzed their effects on RTK phosphorylation via direct Western blotting or Western blotting after immunoprecipitation (IP) with antibodies against p-TRK, p-PDGFRα, and p-RON. The relative phosphorylation of RTKs was calculated in relation to the total amount of RTK detected by densitometry analysis of representative Western blotting (Supplementary Table S5).

The effects of TKIs on TRKA (hereafter referred to as TRK) were analyzed with L-1236 and HDLM-2, which showed that when pretreated with sorafenib or lestaurtinib, β-NGF failed to induce TRK phosphorylation in both cell lines (Fig. 3A, Supplementary Table S5).

In L-428, RON is significantly phosphorylated without application of its ligand, most likely due to the presence of a constitutively active splice variant lacking the fourth extracellular immunoglobulin plexin transcription domain (data not shown) (24). Sorafenib nearly completely abolished the phosphorylation of RON in L-428 (Fig. 3B), and also lestaurtinib had an inhibitory effect on the phosphorylation of RON (Fig. 3B and Supplementary Table S5).

In U-HO1, ligand-induced phosphorylation of PDGFRα was decreased by sorafenib and also by lestaurtinib (Fig. 3C, Supplementary Table S5). Surprisingly, expression and phosphorylation of PDGFRα was increased in KM-H2 cells when treated with lestaurtinib (Fig. 3D), whereas evaluation of the effects of sorafenib in KM-H2 was difficult due to the low PDGFRα phosphorylation under normal culture conditions. After preincubation with lestaurtinib, sorafenib caused a decrease of PDGFRα phosphorylation and protein amount (Fig. 3E).

Taken together, the known targets of sorafenib and lestaurtinib, PDGFRα and TRK, respectively, could be verified as targets in HRS cell lines, whereas TRK and RON were identified as new targets of sorafenib and PDGFRα and RON as new targets of lestaurtinib.

Sorafenib and lestaurtinib reduce activation of multiple intracellular signaling pathways

To analyze the effect of TKIs on signaling pathways usually activated by RTKs, Western blotting was conducted. Treatment with sorafenib caused the reduction of phosphorylation of JAK2, various STATs and RAF1 in all 3 cell lines, and reduction of AKT1 in L-1236 and KM-H2. However, in all instances, the reduced phosphorylation was accompanied by concomitant reduction of total protein amounts (Fig. 4A). Extracellular signal–regulated kinase (ERK)1/2 were less phosphorylated in all cell lines without decreases in total protein amounts.

Lestaurtinib caused decreases in phosphorylation of AKT1, JAK2, and RAF1 in all cell lines with concomitant reduction of RAF1 total protein amount (Fig. 4B). The phosphorylation levels of STATs were reduced in L-428 and L-1236. Unexpectedly, the phosphorylation of ERK1/2 was increased in all 3 cell lines, and the phosphorylation of STATs was constant in KM-H2 after lestaurtinib treatment with elevated total protein amounts of JAK2 and STATs in this cell line.

Sorafenib provokes endoplasmic reticulum stress in HRS cell lines

Sorafenib can cause endoplasmic reticulum (ER) stress with a general reduction of translation and caspase-3–independent cell death in human leukemia cells (25), and ER stress could thus account for the reduction in total protein amounts of several signaling molecules and caspase-3–independent cell death in HRS cell lines. Therefore, we analyzed the effect of sorafenib on different ER stress markers in HRS cell lines by Western blotting and quantitative real-time PCR (qRT-PCR). The phosphorylation of PERK, one of the ER resident proteins inducing the stress response (26), and the expression of genes induced by ER stress, GADD34 and GADD153 (25–27), were monitored (Fig. 5).

All 3 analyzed cell lines had phosphorylated PERK when incubated with solvent alone, which increased upon incubation with 10 μmol/L sorafenib in L-428 and KM-H2. Incubation with 10 μmol/L sorafenib increased GADD34 and GADD135 mRNA levels in all cell lines as determined by qRT-PCR (7.5- to 16-fold and 14- to 37-fold increases for GADD34 and GADD135, respectively). To investigate whether the induction of ER stress by sorafenib was an HRS cell–specific effect, the expression of GADD34 and GADD153 was also analyzed in HDLM-2, U-HO1 and 2 non–Hodgkin lymphoma cell lines with comparable sensitivity toward the TKIs, L-363 and Ramos. In all cell lines, 24-hour incubation with 10 μmol/L sorafenib caused an increase of GADD34 and GADD135 mRNA levels comparable with the effect in HRS cell lines (Supplementary Fig. S1).
These observations indicate that sorafenib provokes ER stress, which may cause cell death, in HRS and the other cell lines tested. The induction of ER stress could thus be a general effect of sorafenib.

**Lestaurtinib activates the alternative NF-κB pathway in HRS cell lines**

Despite the dephosphorylation of the anti-apoptotic AKT1 and in contrast to a recent study (15), lestaurtinib did not induce apoptosis in L-428 and KM-H2 in our hands. The lestaurtinib induced ERK1/2 phosphorylation in all HRS cell lines and increased expression of PDGFRα, JAK2, and STATs in KM-H2 indicated that the expression and activation of several signaling pathway constituents, which could support survival, increased upon AKT1 and JAK inhibition in HRS cell lines. To address this issue systematically, we conducted differential genome-wide gene expression analysis after lestaurtinib treatment of KM-H2.

KM-H2 cells were incubated with 1 μmol/L lestaurtinib or solvent for 24 hours, and differentially expressed transcripts were identified using replicates of Affymetrix GeneChip Human 1.0 Arrays and the GeneSpringGX software.

Figure 1. Sensitivity of Hodgkin-Reed/Sternberg cell lines to TKIs. A, chemical structures of compounds used in this study. B, HRS cell lines L-1236 and L-428 (both expressing TRKA, RON, DDR2, and EPHB1), KM-H2 (expressing PDGFRα and DDR2), HDLM-2 (expressing TRKA, DDR2, and EPHB1), and U-HO1 (expressing PDGFRα but not TRKA and RON) were incubated with different TKIs. Percentage of viable cells after 48-hour treatment with 5 μmol/L TKI are shown as mean values of 2 experiments. Reductions below 80% were considered relevant. For lapatinib, sunitinib, and dasatinib, see Supplementary Table S4.
software. Sixty-three genes were at least 2-fold upregulated and 180 genes were at least 2-fold downregulated (Supplementary Tables S6 and S7). Gene ontology analysis revealed the affiliation of most (116 of 180) downregulated genes to biologic processes linked to cell division and proliferation (Supplementary Table S8), and most of these genes enhance cell-cycle progression. The upregulated genes showed no strong affiliation to specific biologic processes.

We chose 7 upregulated genes that could enhance cell survival (LIPH, PIM2, SOS2, STAT4, TNFSF8, TNFSF10, and TRAF3IP3) for further analysis by qRT-PCR with KM-H2, L-428, and L-1236 (Fig. 6A). For KM-H2, increases in RNA levels of all genes could be confirmed, showing the validity of the microarray analysis. The most distinctly induced genes in all 3 cell lines were TNFSF8 (6- to 18-fold), the CD30 ligand, and TNFSF10 (3- to 13-fold), the TRAIL-R ligand.

CD30, a cell surface TNF receptor (TNFR) expressed by most HRS cells and HRS cell lines (28), has been shown to activate the MEK/ERK pathway and the classical and alternative NF-kB pathways upon binding of its ligand (29–31). The observed increases in ERK1/2 phosphorylation upon lestaurtinib treatment are thus most likely due to the CD30L upregulation. To determine whether the lestaurtinib-induced upregulation of CD30L resulted also in NF-kB activation, we analyzed the expression of transcriptional targets of the classical NF-kB pathway (ICAM1, LTA, and BIRC3) by qRT-PCR, but observed no increases in expression (data not shown). In addition, we analyzed activation of the alternative NF-kB pathway by monitoring the accumulation of p52 by Western blotting. After 24-hour incubation with 1 µmol/L lestaurtinib, increased amounts of p52 were detected in all 3 cell lines (Fig. 6B).

To investigate whether the lestaurtinib-induced upregulation of CD30L was a more general mechanism, 2 further HRS and anaplastic large cell lymphoma (ALCL) cell lines expressing also CD30 were analyzed by qRT-PCR for CD30L mRNA changes after 24-hour treatment with 1 µmol/L lestaurtinib (Supplementary Fig. S2). An increase of CD30L mRNA amounts could be observed in one of the HRS cell lines but not in the second HRS cell line, nor in the ALCL cell lines, indicating that the upregulation of CD30L is a HRS cell–specific resistance mechanism.

Taken together, lestaurtinib induced upregulation of CD30L and TRAIL in HRS cell lines and the former most likely caused ERK1/2 phosphorylation and activation of the alternative NF-kB pathway.
TKIs can increase the effect of conventional chemotherapeutic drugs

The combination of conventional chemotherapeutic drugs with TKIs is a strategy to enhance the effect of cancer therapy (32). For example, the EGFR inhibitor erlotinib is added to conventional chemotherapy for patients with advanced-stage pancreatic cancer (33). Therefore, we examined the effect of sorafenib and lestaurtinib on HRS cell lines in combination with drugs used in conventional Hodgkin lymphoma chemotherapy.

The metabolic activity of L-428, L-1236, and KM-H2 cells was measured (MTT assay) after 72-hour treatment with vinblastine and doxorubicin alone, sorafenib or lestaurtinib alone, or the combination of one chemotherapeutic with one TKI (Supplementary Fig. S3). The inhibitory effects of vinblastine and doxorubicin were in general enhanced by the simultaneous treatment with sorafenib or lestaurtinib compared with either TKI or vinblastine or doxorubicin alone. This effect was most evident for the combination of sorafenib with either conventional chemotherapeutic drug in L-428 and KM-H2 cells.

Discussion

Several different RTKs are expressed and activated in HRS cells of Hodgkin lymphoma (5), and the inhibition of these RTKs with TKIs could be a novel option for...
treatment. In a screening of 9 TKIs, we found that all HRS cell lines tested were sensitive toward the TKIs sorafenib and lestaurtinib at concentrations achievable in patients. Sorafenib, a multikinase inhibitor, induced cell death of L-428, L-1236, and KM-H2 after prolonged incubation. Although other studies investigating sorafenib observed antiproliferative activity at low nanomolar concentrations when tumor cell lines with mutation activated RTKs were examined (34), the sensitivities observed for HRS cell lines were comparable with what we found for other cell lines and what was found in other studies, analyzing for example carcinoma or other lymphoma cell lines (19–21), and is in line with a recently published study analyzing the effect of the TKI on HRS cell lines (14).

Sorafenib inhibited not only the activation of its known targets RAF1 and PDGFR\(_a\) (35, 36) but also TRK and RON and thus at least 2 of the kinases tested here in each HRS cell line, and, given its broad effects on several different cell lines, likely also other kinases not tested here.

The sorafenib-induced deactivation of intracellular signaling molecules was often accompanied by losses of total amounts of these proteins, and sorafenib can cause ER stress with reduced translation in leukemia cells (25). As we observed typical features of ER stress also in the

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**Figure 3.** Western blot analysis of RTK phosphorylation after incubation with sorafenib or lestaurtinib. A, Western blot analysis of immunoprecipitated RTK after 4-hour incubation of cells in 1% fetal calf serum to increase induction of RTK phosphorylation, 10-minute preincubation with TKIs sorafenib (10 \(\mu\)mol/L) or lestaurtinib (1 \(\mu\)mol/L) and stimulation with 25 ng/mL \(\beta\)-NGF for 2 minutes. B, Western blot analysis of immunoprecipitated RON after 24-hour treatment with 10 \(\mu\)mol/L sorafenib or 1 \(\mu\)mol/L lestaurtinib. C, Western blot analysis of PDGFR\(_a\) after cells were serum-starved and pretreated with sorafenib or lestaurtinib as described for A and stimulated with 100 ng/mL PDGFAA for 2 minutes. D, Western blot analysis of PDGFR\(_a\) in KM-H2 after 24-hour incubation with 1 \(\mu\)mol/L lestaurtinib or 10 \(\mu\)mol/L sorafenib. E, Western blot analysis of PDGFR\(_a\) in KM-H2 cells pretreated with 1 \(\mu\)mol/L lestaurtinib for 24 hours and subsequent incubation with 10 \(\mu\)mol/L sorafenib for 10 minutes or 1 hour. All experiments were carried out at least twice.
sorafenib-treated HRS cell lines, it is likely that the losses of total amounts of signaling molecules are due to ER stress induced reduction in translation combined with their fast turnover rates. Furthermore, ER stress caused cell death in a caspase-3–independent way in leukemia cells (25) and also the sorafenib induced cell death in L-428 and KM-H2 was independent of caspase-3 activation. We conclude that cell death in L-428 and KM-H2 might be induced by the same ER stress–dependent mechanisms as in leukemia cells (25). As induction of ER stress markers in response to sorafenib treatment was also observed in 2 other non–Hodgkin lymphoma cell lines, induction of ER stress by sorafenib may be a more general phenomenon.

The sensitivity of HRS cells toward lestaurtinib is comparable with what was reported for acute myeloid leukemia cells dependent on mutant FLT3 activity (22) or myeloproliferative neoplasm cell lines with the constitutively active JAK2-mutant V617F (23). However, also all other lymphoma, leukemia, and carcinoma cell lines tested here were even more sensitive than the HRS cell lines, which could be either due to the interaction of lestaurtinib with a broad kinase target spectrum, as recently supposed (37), or dependency of all tested cell lines on JAK/STAT signaling. Lestaurtinib was recently reported to induce caspase-3 activity in some HRS cell lines (15). Although exhibiting strong cytostatic effects, we observed an
induction of apoptosis by lestaurtinib only in the already preapoptotic L-1236, in contrast to the caspase-3 activation in L-428 observed by Diaz and colleagues (15).

Lestaurtinib inhibited activation of its known targets TRK and JAK2 and phosphorylation of AKT1 in all and phosphorylation of several STATs in L-428 and L-1236. Furthermore, it inhibited ligand-induced phosphorylation of PDGFRα in one cell line and reduced the constitutive phosphorylation of RON in L-428. In KM-H2, however, lestaurtinib treatment caused increases in total amounts of JAK2 and STATs, and the amounts of phosphorylated STAT3 and 5 remained constant, despite complete JAK2 dephosphorylation. We observed also increases in expression and phosphorylation of PDGFRα in KM-H2 upon lestaurtinib treatment, and thus it is conceivable that the activated PDGFRα directly or via activation of other intracellular tyrosine kinases, for example, SRC family kinases, contributed to the STAT phosphorylation under lestaurtinib treatment (38). Such maintenance of activation of a signaling pathway on which tumor cells are “dependent” by increased expression and activation of another tyrosine kinase upon pharmacologic inhibition of the “driver” tyrosine kinase is a known mechanism of secondary TKI resistance.

Another way of tumor cells to escape the pharmacologic inhibition of antiapoptotic signaling pathways is the activation of alternative pathways (39–41). As lestaurtinib treatment caused deactivation of AKT1, whose activity is usually essential for HRS cell survival (42, 43), without inducing apoptosis, we conducted a systematic screen for upregulated constituents of other signaling pathways and found that lestaurtinib caused significant increases of CD30L and TRAIL expression in all 3 analyzed HRS cell lines.

The activation of CD30 by CD30L has previously been shown to cause ERK phosphorylation and NF-κB activation in HRS cell lines (29–31), and, indeed, we observed increased ERK1/2 phosphorylation and increases in the activity of the alternative NF-κB pathway upon lestaurtinib treatment in all analyzed HRS cell lines. TRAIL usually induces apoptosis in tumor cells via the TRAIL-R–associated DISC. However, in HRS cells, apoptosis induction via TRAIL-R–associated caspase-8 is prevented by the strong c-FLIP expression (44, 45), and in cancer cells resistant toward TRAIL-induced apoptosis, TRAIL increased survival in a NF-κB–dependent fashion (46). It is thus conceivable that in HRS cell lines, the lestaurtinib induced increases in TRAIL expression also contribute to NF-κB activation. Furthermore, activation of the alternative NF-κB pathway may also be increased by the observed TRAF3IP3 upregulation, which by releasing NIK from TRAF3 may cause activation of IKKα with subsequent p100 processing (47, 48).

It is thus likely that in HRS cells, the inhibitory effects of lestaurtinib on the survival enhancing AKT1 are compensated by activation of CD30 signaling via CD30L...
upregulation and activation of ERK1/2 and NF-κB and also by TRAIL-mediated NF-κB activation. As the combined application of the MEK/ERK inhibitor PD0325901 with lestaurtinib did not result in apoptosis (data not shown), activation of NF-κB pathway may be the more relevant mechanism of apoptosis prevention. Interestingly, this effect seems to be HRS cell–specific, as no lestaurtinib-induced upregulation of CD30L transcription was observed in other CD30-expressing cell lines.

The addition of TKIs to cancer treatment with conventional chemotherapeutic drugs has beneficial effects in several instances. Therefore, we combined lestaurtinib and sorafenib with doxorubicin and vinblastine and indeed observed increased inhibitory effects on metabolic activity compared with the single agents.

The combination of conventional chemotherapeutic drugs with sorafenib showed additive inhibitory effects in those cell lines possibly undergoing completely caspase-3–independent cell death in response to sorafenib, L-428 and KM-H2. Apoptosis via caspase-3 is one major cell death pathway caused by conventional chemotherapeutic agents (49). Potentially, the combination of the 2 drugs is most efficient when different mechanisms of cell death are triggered, the apoptotic pathway through caspase-3 by a conventional chemotherapeutic agent and caspase-independent cell death by sorafenib.

Combining lestaurtinib with conventional chemotherapeutic drugs did not significantly alter the effect of lestaurtinib alone in L-428 and KM-H2. Here, lestaurtinib-mediated NF-κB activation may provide protection from moderate concentrations of conventional chemotherapeutics.

In summary, we show here that lestaurtinib inhibits proliferation, and sorafenib induces cell death in several HRS cell lines. The sorafenib-induced cell death was caspase-3-independent, in line with the induction of ER stress, which can cause cell death via caspase-3–independent mechanisms. The combination of sorafenib with conventional chemotherapeutics had additive effects, likely due to the induction of cell death by 2 different mechanisms. This indicates a potential use of sorafenib in Hodgkin lymphoma therapy in combination with conventional chemotherapeutic drugs. Regarding the effects of lestaurtinib, our results indicate that HRS cells can escape apoptosis by TKI-mediated AKT1 inhibition via increases in the TNFR-mediated activation of the alternative NF-κB pathway. This mode of TKI resistance differs from the usually observed upregulation of other tyrosine kinases or increased signaling via other tyrosine kinase-activated pathways and seems to be an HRS cell–specific resistance mechanism.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
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Induction of Endoplasmic Reticulum Stress by Sorafenib and Activation of NF-κB by Lestaurtinib as a Novel Resistance Mechanism in Hodgkin Lymphoma Cell Lines

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