p185$^{BCR/ABL}$ has a lower sensitivity than p210$^{BCR/ABL}$ to the allosteric inhibitor GNF-2 in Philadelphia chromosome-positive acute lymphatic leukemia

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ABSTRACT

Background

The t(9;22) translocation leads to the formation of the chimeric *breakpoint cluster region/c-abl oncogene 1* (*BCR/ABL*) fusion gene on der22, the Philadelphia chromosome. The p185$^{BCR/ABL}$ or the p210$^{BCR/ABL}$ fusion proteins are encoded as a result of the translocation, depending on whether a "minor" or "major" breakpoint occurs, respectively. Both p185$^{BCR/ABL}$ and p210$^{BCR/ABL}$ exhibit constitutively activated ABL kinase activity. Through fusion to BCR the ABL kinase in p185$^{BCR/ABL}$ and p210$^{BCR/ABL}$ "escapes" the auto-inhibition mechanisms of c-ABL, such as allosteric inhibition. A novel class of compounds including GNF-2 restores allosteric inhibition of the kinase activity and the transformation potential of BCR/ABL. Here we investigated whether there are differences between p185$^{BCR/ABL}$ and p210$^{BCR/ABL}$ regarding their sensitivity towards allosteric inhibition by GNF-2 in models of Philadelphia chromosome-positive acute lymphatic leukemia.

Design and Methods

We investigated the anti-proliferative activity of GNF-2 in different Philadelphia chromosome-positive acute lymphatic leukemia models, such as cell lines, patient-derived long-term cultures and factor-dependent lymphatic Ba/F3 cells expressing either p185$^{BCR/ABL}$ or p210$^{BCR/ABL}$ and their resistance mutants.

Results

The inhibitory effects of GNF-2 differed constantly between p185$^{BCR/ABL}$ and p210$^{BCR/ABL}$ expressing cells. In all three Philadelphia chromosome-positive acute lymphatic leukemia models, p210$^{BCR/ABL}$-transformed cells were more sensitive to GNF-2 than were p185BCR/ABL-positive cells. Similar results were obtained for p185$^{BCR/ABL}$ and the p210$^{BCR/ABL}$ harboring resistance mutations.

Conclusions

Our data provide the first evidence of a differential response of p185$^{BCR/ABL}$- and p210$^{BCR/ABL}$- transformed cells to allosteric inhibition by GNF-2, which is of importance for the treatment of patients with Philadelphia chromosome-positive acute lymphatic leukemia.

Key words: Philadelphia chromosome, BCR/ABL, allosteric inhibition, acute lymphatic leukemia, molecular therapy.

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Introduction

The der22 of t(9;22)(q34;q11), the so-called Philadelphia chromosome (Ph), is detected in 95% of patients with chronic myeloid leukemia, as well as in 20-30% of adult acute lymphatic leukemia (ALL) patients. Chronic myeloid leukemia is a myeloproliferative syndrome characterized by an indolent chronic phase with an overgrowth of the mature myeloid cell population, which is, if not treated, inevitably followed by an acute phase, the so-called blast crisis. Clinically, the blast crisis resembles acute leukemia, with a poor prognosis and resistance to therapy.[1-3] Chronic myeloid leukemia in blast crisis displays a myeloid phenotype in two-thirds of cases and a lymphatic phenotype in the remaining one-third.[4] In contrast, Ph$^+$ ALL is an acute disease from the onset and is characterized by blasts blocked at the pre-lymphatic stage of differentiation. Patients suffering from Ph$^+$ ALL constitute a high-risk group.[5] Lymphatic blast crisis and Ph$^+$ ALL are considered equivalent.[1-3,6]

On chromosome 22, the t(9;22) involves the *Breakpoint Cluster Region* (*BCR*) gene locus. Two principal breaks occur: the *M-bcr*, between exons 12 and 16 leading to the creation of p210$^{BCR/ABL}$, which is the hallmark of chronic myeloid leukemia and the *m-bcr*, which maps to the first intron of *BCR* and leads to the creation of p185$^{BCR/ABL}$. The breakpoint on chromosome 9 is constantly located in intron 1 of the *ABL* gene locus.[4] p185$^{BCR/ABL}$ is exclusive to the Ph$^+$ ALL, whereas p210$^{BCR/ABL}$ is found in about 30% of Ph$^+$ ALL as well as in chronic myeloid leukemia.[4] p210$^{BCR/ABL}$ differs from p185$^{BCR/ABL}$ by the presence of the putatively oncogenic Rho-GEF domain. Nevertheless, only few functional and biological differences between p210$^{BCR/ABL}$ and p185$^{BCR/ABL}$ are known.[7] Both exhibit constitutively activated kinase activity responsible for the induction of the leukemic phenotypes.[5,8] Inhibition using imatinib mesylate (imatinib), nilotinib or dasatinib, classical ATP competitors, is a valid concept for the causal therapy of Ph$^+$ leukemia.

The rapid acquisition of therapy resistance by patients with advanced Ph$^+$ lymphatic leukemia, together with frequent features of patients, such as age or adverse side effects, justify the need for novel approaches to the molecular therapy of these diseases.[9] One such novel approach is the restoration of the allosteric inhibition of ABL-kinase activity, one of the major auto-inhibitory mechanisms of the ABL-kinase which is lost by fusion to BCR. Allosteric inhibition occurs through the binding of the myristoylated N-terminus (exon 1) to a hydrophobic pocket in the kinase domain, the myristoyl binding pocket, followed by conformational changes that allow the intra-molecular docking of the SH2 domain to the kinase domain. This process, called "capping", leads to an auto-inhibited conformation of c-ABL. The lack of the "cap region" in exon 1 allows BCR/ABL to "escape" auto-inhibition.[10,11]

In this study, we aimed to further develop allosteric inhibition in Ph$^+$ ALL by investigating the anti-proliferative activity of GNF-2 in different Ph$^+$ ALL models.

Design and Methods

Plasmids

The cDNA encoding p185$^{BCR/ABL}$, p210$^{BCR/ABL}$ p185$^{BCR/ABL}$ Y253F, p185$^{BCR/ABL}$ E255K, and p185$^{BCR/ABL}$ T315I have been described previously.[15] The p210$^{BCR/ABL}$ Y253F, p185$^{BCR/ABL}$ E255K, and p185$^{BCR/ABL}$ T315I were obtained by transfer of a KpnI fragment from p185$^{BCR/ABL}$ E255K, and p185$^{BCR/ABL}$ T315I mutants to a KpnI digested p210$^{BCR/ABL}$ in the pEntry vector (Gateway-Invitrogen, Karsruhe, Germany). The resulting p210$^{BCR/ABL}$ mutant sequences were then recombined in the PAULO destination vector by a Gateway reaction according to the manufacturer's instructions (Invitrogen). All retroviral expression vectors used in this study were based on the bi-cistronic vector PAULO.[12]

Cell lines

The Ba/F3, BV-173, Tom-1 and Nalm-6 cells were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and were maintained as previously described. Long-term cultures of cells derived from Ph$^+$ ALL patients (PD-LTC) were maintained in a serum–free medium consisting of Iscove's modified Dulbecco's medium (IMDM) supplemented with 1 mg/mL of bovine insulin, 5×10^{-5} M β–mercaptoethanol (Sigma, Steinheim, Germany), 200 mg/mL Fe$^+$–saturated human apo–transferrin (Invitrogen, Karlsruhe, Germany), 0.6% human serum albumin (Sanquin, Amsterdam, the Netherlands), 2.0 mM L–glutamine and 20 mg/mL cholesterol (Sigma).[13] Transfection and retroviral infection were performed as previously described.[12] GNF-2 was dissolved in dimethylsulfoxide (DMSO) (Sigma) for 1000 x stock solutions (50 μM, 100 μM, 150 μM, 250 μM, 300 μM, 500 μM, and 1 mM).

Cytotoxicity/proliferation

Cytotoxicity/proliferation was assessed using the XTT proliferation kit according to the manufacturer's instructions (Roche, Mannheim, Germany). Cell growth was assessed by dye exclusion using trypan-blue. The IC_{50} was calculated using Erithacus software (Erithacus Ltd. East Grinstead, UK).

Western blotting

Western blotting was performed accordingly to widely established protocols using the following antibodies: anti-ABL (α-ABL) (St. Cruz Biotechnology, Santa Cruz, USA), anti-phospho-Y245 ABL (α-p-ABL-Y245), anti-CRKL (α-CRKL), and anti-phosphorylated CRKL (α-p CRKL)(Cell Signaling, Boston, USA).

Soft agar assay

PD-LTC (10^4 cells) were suspended in 1 mL "top-agar", 0.25% bacto-agar (DIFCO Laboratories, Detroit, USA) in IMDM and stacked on 0.5% bacto-agar in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS) in six-well plates. Colonies were counted after incubation for 15 days.

Results

Philadelphia chromosome-positive acute lymphatic leukemia cell lines exhibit a differential response to GNF-2 which correlates with the expression of p185$^{BCR/ABL}$ or p210$^{BCR/ABL}$

To investigate the selective activity of the allosteric inhibitor GNF-2 on Ph$^+$ ALL, we compared the human patient-derived cell lines Tom-1 and BV-173 expressing p185$^{BCR/ABL}$ and p210$^{BCR/ABL}$, respectively. Ph$^-$ Nalm-6 cells were used as negative controls. These cell lines have a nearly identical pre-B lymphatic differentiation level. Cytotoxicity was assessed by the XTT and dye exclusion assays. Here we show that GNF-2 inhibited proliferation of BV-173 and Tom-1 cells with an IC_{50} of 125 nM and 500

nM, respectively, without affecting the Nalm-6 cells (Figure 1A). The growth of BV-173 and Tom-1 was blocked completely at concentrations of 0.25 μM and 1 μM, respectively, whereas no effect was observed in Nalm-6 cells (Figure 1B and *data not shown*). The differences in response to GNF-2 were not due to differences in the expression levels of BCR/ABL between the Ph+ cell lines (Figure 1C). In fact, the BV-173 cells expressed a higher level of BCR/ABL with a stronger basic autophosphorylation, as compared to Tom-1, whereas the effect of GNF-2 on autophosphorylation was more pronounced in the BV-173 cells than in the Tom-1 cells. In contrast, the effects of GNF-2 on substrate phosphorylation (CRKL) seemed to be more pronounced in the p185$^{BCR/ABL}$-positive Tom-1 cells than in the BV-173 cells (Figure 2C). Similar results were obtained with other Ph+-positive ALL cell lines, such as SupB15 (*data not shown*).

Taken together, these data strongly suggest a different response of Ph+-positive ALL cell lines to GNF-2 treatment in accordance to the expression of either the p185$^{BCR/ABL}$ or p210$^{BCR/ABL}$ fusion protein.

Philadelphia chromosome-positive patient-derived long-term culture cells expressing p210$^{BCR/ABL}$ are more responsive to GNF-2 than those expressing p185$^{BCR/ABL}$

Ph+ ALL in adults is not fully represented by cell lines. We, therefore, compared the response of PD-LTC from Ph+ ALL patients to GNF-2 in relationship to the cells' expression of p210$^{BCR/ABL}$ and p185$^{BCR/ABL}$. The PD-LTC were directly derived from bone marrow cells of Ph+ ALL patients cultured in a specific culture medium.[13] We compared the responses from increasing concentrations of PD-LTC of two Ph+ ALL patients expressing p185$^{BCR/ABL}$ (BV and PH) with those of two patients expressing p210$^{BCR/ABL}$ (CM and VB). As negative controls, we used the PD-LTC of a Ph- ALL patient (HP). Cytotoxicity/proliferation was assessed at 72 h by XTT, as described above. GNF-2 inhibited the proliferation of both p210$^{BCR/ABL}$- and p185$^{BCR/ABL}$-expressing PD-LTC, but with different IC$_{50}$ values of 75-100 and 400-1000 nM, respectively, and did not affect the proliferation of cells from the Ph- ALL patient (Figure 2A). The PD-LTC were able to form colonies in the semi-solid medium, demonstrating their transformed phenotype (Figure 2B and *data not shown*). The colony-forming potential of p210$^{BCR/ABL}$-positive PD-LTC, but not of p185$^{BCR/ABL}$-positive PD-LTC, was significantly reduced by 250 nM and 500 nM of GNF-2. The differential response was closely related to the variation in inhibition of BCR/ABL kinase activity. GNF-2 only slightly inhibited the autophosphorylation of p185$^{BCR/ABL}$ in cells from the two Ph+ ALL patients expressing p185$^{BCR/ABL}$ (BV and PH), whereas kinase activity was nearly abolished in the p210$^{BCR/ABL}$-expressing PD-LTC (from patients CM and VB), even at low concentrations. In contrast to the substrate phosphorylation of CRKL, which was only slightly affected in p210$^{BCR/ABL}$-positive but not in p185$^{BCR/ABL}$-positive PD-LTC, that of BCR and STAT5 was reduced in both but to a greater extent in p210$^{BCR/ABL}$ -positive PD-LTC (Figure 2C).

In summary, these data show that Ph+ PD-LTC expressing p210$^{BCR/ABL}$ are more sensitive to GNF-2 than are those expressing p185$^{BCR/ABL}$.

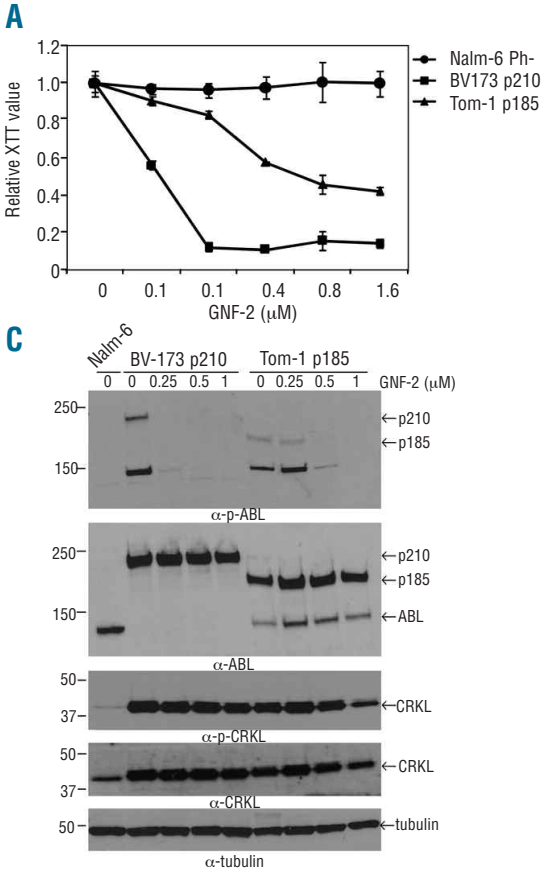

Figure 1. Effects of GNF-2 on Ph+ cell lines expressing either p185$^{BCR/ABL}$ or p210$^{BCR/ABL}$ (A) XTT assay for Ph+ cells expressing p185$^{BCR/ABL}$ or p210 $^{BCR/ABL}$ upon exposure to 0.25, 0.5 and 1 μM GNF-2. The mean ± SD of triplicates from one representative experiment out of three performed is given. (B) The number of viable cells was determined daily by trypan blue dye exclusion. Data represent the mean ± SD of three independent experiments. (C) Western blot analysis of Ph+ cells using antibodies directed against the indicated proteins. Molecular mass references (KDa) are presented. α-tubulin was used as a loading control.

The BCR/ABL fusion proteins mediate differential sensitivity to GNF-2

To examine whether the differential effects of GNF-2 on Ph+ cell lines and PD-LTC are mediated by BCR/ABL and to avoid the bias of yet unknown common or differing features of Ph+ ALL cells, we compared the effect of GNF-2 on factor-dependent Ba/F3 cells, which become factor-independent through the expression of either $p185^{BCR/ABL}$ or $p210^{BCR/ABL}$. Equal expression levels of $p185^{BCR/ABL}$ and $p210^{BCR/ABL}$ were controlled by western blotting excluding differences in transgene expression (Figure 3A). We exposed $p185^{BCR/ABL}$- and $p210^{BCR/ABL}$-expressing Ba/F3 cells to increasing concentrations of GNF-2. Cytotoxicity/proliferation and growth were assessed by XTT and dye exclusion assays. The growth of $p185^{BCR/ABL}$-expressing cells was reduced to a maximum of 50%, even at 2 μM of GNF-2; $p210^{BCR/ABL}$-expressing cell growth, on the other hand, was almost abolished at only 250 nM (Figure 3B and C).

The different sensitivity of $p185^{BCR/ABL}$ and $p210^{BCR/ABL}$ in Ba/F3 cells to GNF-2 correlated with a different rate of inhibition of the autophosphorylation. GNF-2 slightly interfered with substrate phosphorylation of CRKL only in $p210^{BCR/ABL}$ -positive Ba/F3 cells (Figure 3D).

In summary, these data show that the differential sensitivity is mediated by the BCR/ABL fusion proteins $p185^{BCR/ABL}$ and $p210^{BCR/ABL}$.

BCR/ABL resistance mutants mediate differential sensitivity to GNF-2

The major clinical challenge in Ph+ leukemia is drug resistance, which is mainly due to the acquisition by BCR/ABL of point mutations such as the "P-loop" mutations Y253F and E255K or the "gatekeeper" mutation T315I. We, therefore, investigated whether the differential sensitivity to GNF-2 is exhibited also by $p185^{BCR/ABL}$ and $p210^{BCR/ABL}$ harboring the Y253F, E255K or T315I point mutations. To do this we compared the effect of GNF-2 on Ba/F3 cells which became factor-independent through the expression of $p185^{BCR/ABL}$ or $p210^{BCR/ABL}$ resistance mutants. We exposed the cells to increasing concentrations of GNF-2. Cytotoxicity/proliferation and growth were assessed by XTT and dye exclusion assays. For clarity cell counts at day 3 of exposure are represented by bar graphs (Figure 4). We found that the "P-loop" mutants of both $p185^{BCR/ABL}$ and $p210^{BCR/ABL}$ responded to GNF-2 whereas the "gatekeeper"

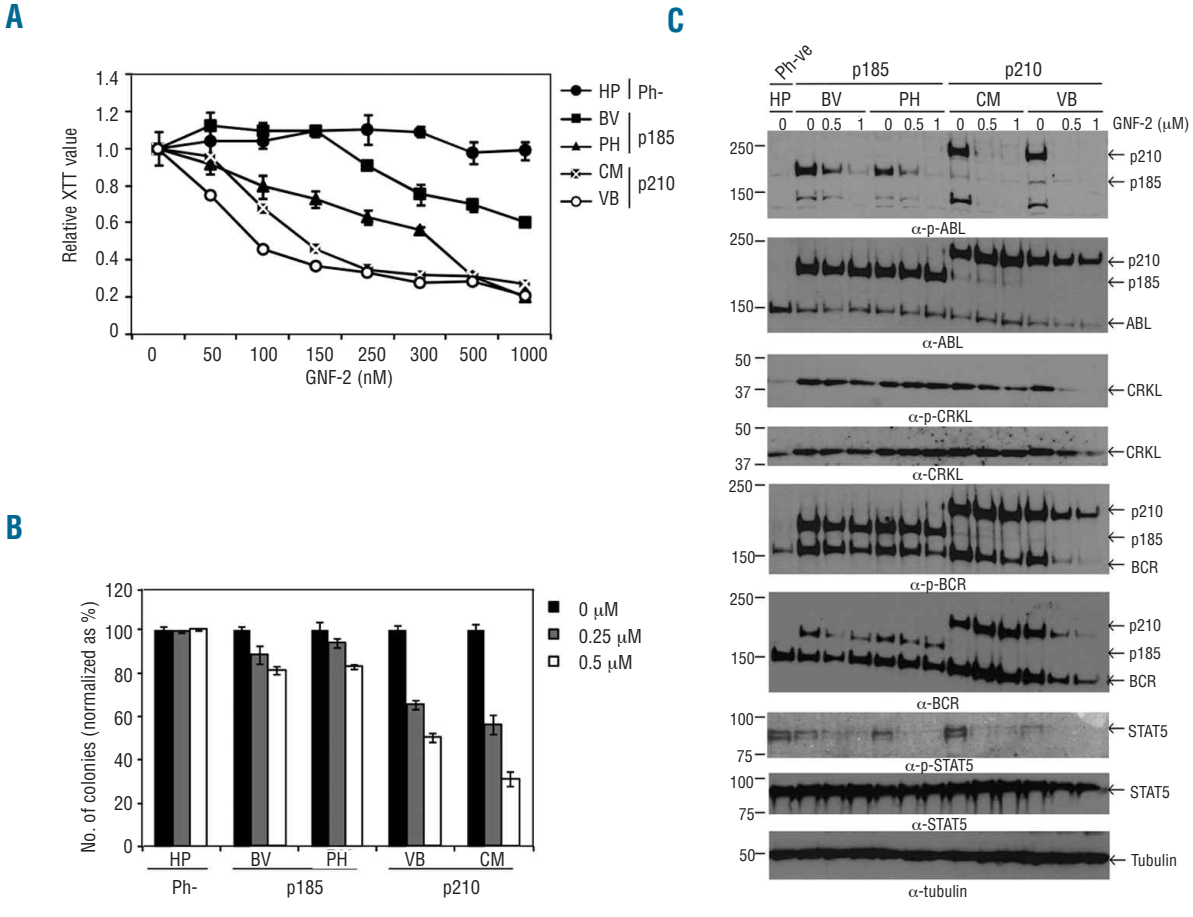

Figure 2. Effects of GNF-2 on PD-LTC of Ph+ ALL patients (A) XTT assay for PD-LTC cells expressing $p185^{BCR/ABL}$ or $p210^{BCR/ABL}$ upon exposure to 0.25, 0.5 and 1 μM GNF-2. The means ± SD of triplicates from one representative experiment out of three performed are given. (B) Soft agar assay: PD-LTC expressing $p185^{BCR/ABL}$ or $p210^{BCR/ABL}$ were seeded at 10^4 cells/well in soft-agar in six-well-plates. After 15 days, the colonies were counted, and the means ± SD of triplicates of one representative of two experiments performed are given. (C) Western blot analysis of PD-LTC using antibodies directed against the indicated proteins.

mutant T315I was completely refractory to GNF-2 (Figure 4A-C). p210$^{BCR/ABL}$ P-loop mutants showed a clearly greater sensitivity to GNF-2 than their p185$^{BCR/ABL}$ counterparts. Equal expression levels of p185$^{BCR/ABL}$ and p210$^{BCR/ABL}$ mutants were controlled by western blotting, excluding differences in transgene expression (Figure 4 A-C and *data not shown*).

Taken together these data suggest that p210$^{BCR/ABL}$ P-loop resistance mutants are more sensitive to allosteric inhibition by GNF-2 than are those of p185$^{BCR/ABL}$, whereas the "gatekeeper" mutation T315I confers complete resistance independently of the BCR/ABL fusion protein.

Discussion

Allosteric inhibition is a novel approach for targeting BCR/ABL. The aim of this study was to explore the efficacy and the clinical feasibility of allosteric inhibition in the treatment of Ph+ ALL. We investigated the effects of GNF-2 on both p185$^{BCR/ABL}$- and p210$^{BCR/ABL}$-positive ALL models. We found that the allosteric inhibitor GNF-2, at clinically feasible concentrations, effectively suppresses growth of Ph+ ALL cells. In all models the p210$^{BCR/ABL}$-positive cells were more sensitive to GNF-2 than were the p185$^{BCR/ABL}$-

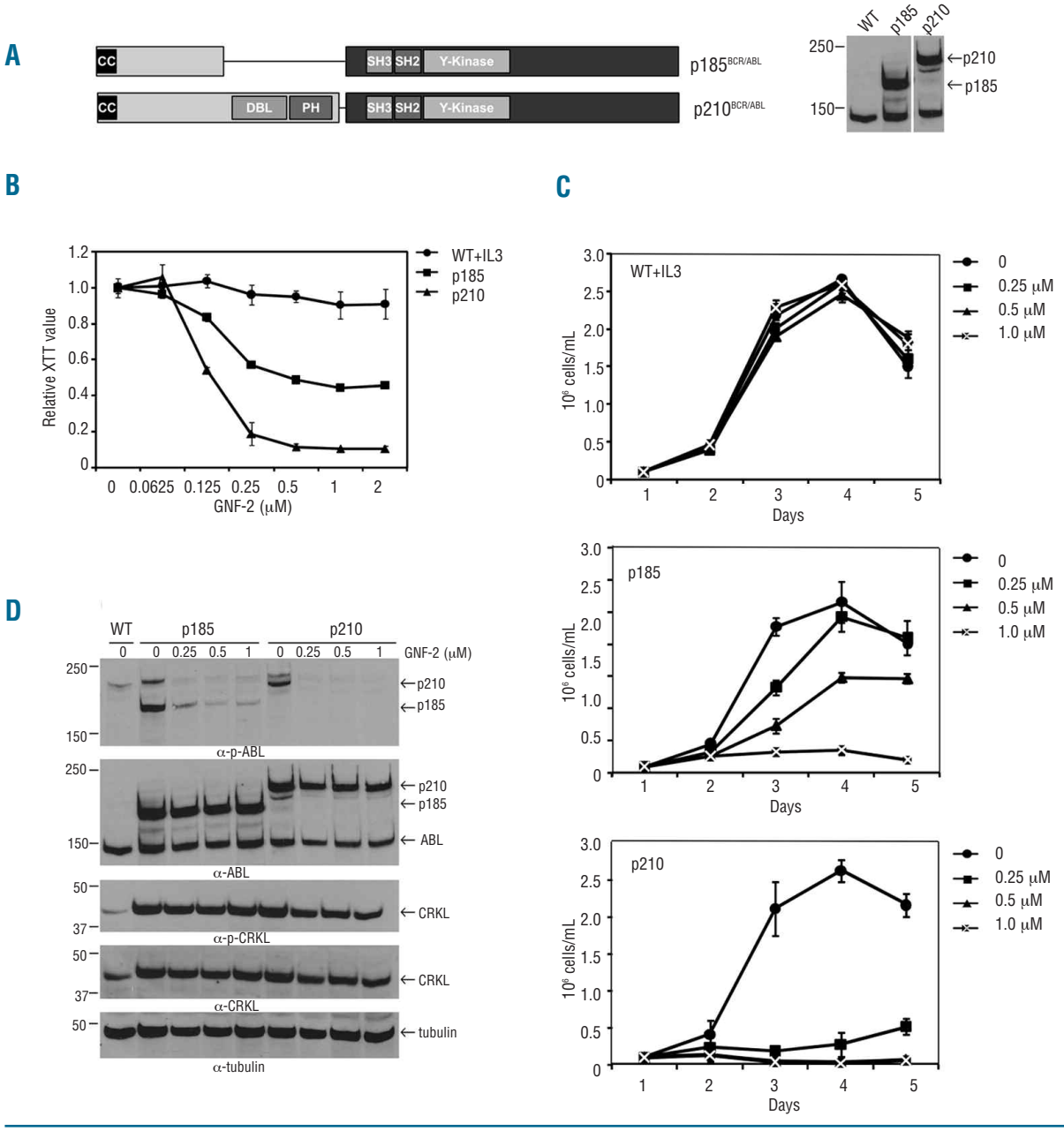

Figure 3. Effects of the allosteric inhibitor (GNF-2) on Ba/F3 cells expressing either p185$^{BCR/ABL}$ or p210$^{BCR/ABL}$ (A) Modular organization of the p185$^{BCR/ABL}$ and p210 $^{BCR/ABL}$ and expression of the transgenes in Ba/F3 cells. (B) XTT assay for Ba/F3 cells expressing p185$^{BCR/ABL}$ or p210 $^{BCR/ABL}$ upon exposure to 0.25, 0.5 and 1 μM GNF-2. The means ± SD of triplicates from one representative experiment out of three performed are given. (C) For factor-independent growth, the number of viable cells was determined daily by trypan blue dye exclusion. The means ± SD of three independent experiments are given. (D) Western blot analysis of Ba/F3 cells expressing the indicated transgenes using antibodies directed against the indicated proteins.

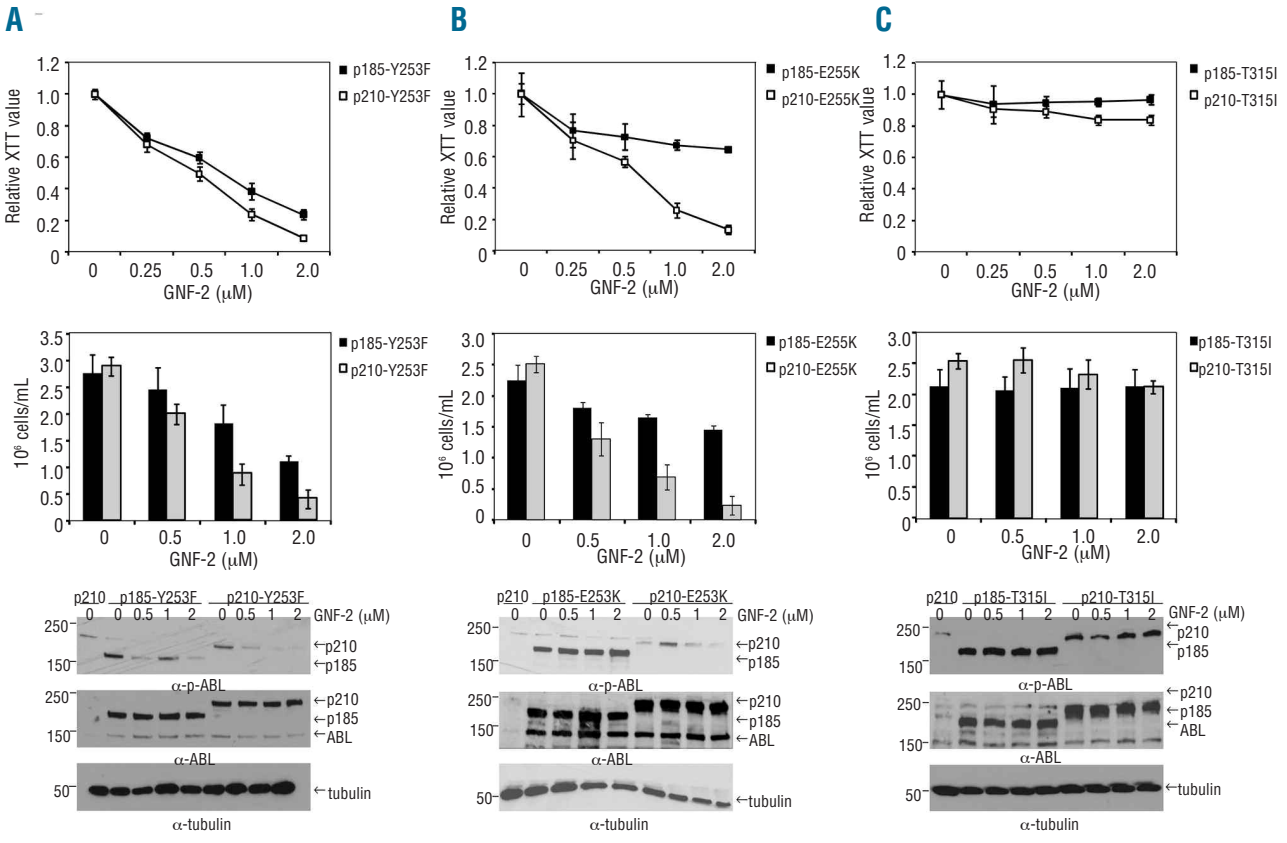

Figure 4. Effects of the allosteric inhibitor (GNF-2) on Ba/F3 cells expressing either $p185^{BCR/ABL}$ or $p210^{BCR/ABL}$ haboring resistance mutations. Ba/F3 cells expressing $p185^{BCR/ABL}$ or $p210^{BCR/ABL}$ harboring either the Y253F (A) the E255K (B) or the T315I mutation (C) were exposed to 0.25, 0.5, 1.0 and 2 μM GNF-2. XTT assays are shown in the upper panels. The means ± SD of triplicates from one representative experiment out of three performed are given. For factor-independent growth, the number of viable cells was determined at day 3 by trypan blue dye exclusion. The means ± SD of three independent experiments are given (middle panels). Western blot analysis of Ba/F3 cells expressing the indicated transgenes using antibodies directed against the indicated proteins (lower panels).

positive cells.

GNF-2 and its analogs are non-ATP competitive ABL kinase inhibitors, which bind to the myristoyl binding pocket in the kinase domain. It seems that the binding of GNF-2 to this pocket stabilizes the protein in an inhibited conformation,[14] resulting in a structural reorganization of ABL that disrupts the catalytic machinery located in the ATP-binding region.[14] This is in accordance with our recent findings that the inhibition of oligomerization of BCR/ABL - monomeric BCR/ABL is inactive - increases the effects of GNF-2.[15] Thus, one can speculate that the variable sensitivities towards GNF-2 may be attributed to differences in the overall conformations between $p185^{BCR/ABL}$ and $p210^{BCR/ABL}$ due to the differences in the BCR portion of the fusion proteins. These differences seem to be maintained also in resistance mutants, as suggested by the greater sensitivity of the $p210^{BCR/ABL}$ forms as compared to their $p185^{BCR/ABL}$ counterparts. In this case, GNF-2 may have a different affinity for the myristoyl binding pocket. Fusion partner influences on the allosteric inhibition of ABL have been shown for another ABL fusion protein; the TEL/ABL is able to confer factor-independent growth through aberrant ABL-kinase activity but did not respond to GNF-2.[11] The reason for the resistance of the T315I forms remains very unclear, even if the homo-oligomerization or the hetero-oligemrization with BCR seems to play a decisive role.[15]

The different sensitivity of $p185^{BCR/ABL}$- and $p210^{BCR/ABL}$-positive Ph^+ ALL cells is specific for GNF-2, because no difference was seen between BV-173 and Tom-1 regarding their high sensitivity to imatinib (*data not shown*). On the other hand the $p185^{BCR/ABL}$-positive PD-LTC (from patients BV and PH) differ for their response to imatinib but not for that to GNF-2 ([15] and *data not shown*).

The differential response of $p185^{BCR/ABL}$- and $p210^{BCR/ABL}$-positive Ph^+ ALL cells to allosteric inhibition can also be seen as an expression of functional and biological differences most likely due to qualitative or quantitative differences in the kinase activity between $p185^{BCR/ABL}$ and $p210^{BCR/ABL}$, as previously shown.[8,16,17] BCR/ABL kinase activity is not characterized only by the autophosphorylation, but also by phosphorylation of substrates, such as CRKL, STAT5 and BCR. It seems that GNF-2 is able to dissociate features of the BCR/ABL kinase activity, because it interferes with autophosphorylation but not with the phosphorylation of all substrates to the same extent. These effects may explain why GNF-2 only blocks proliferation but does not induce apoptosis in BCR/ABL-transformed cells.[11,15] If this is the case, the higher basic kinase activity of $p185^{BCR/ABL}$ may be responsible for its lower sen-

sitivity to allosteric inhibition as compared to $p210^{BCR/ABL}$.

Our data presented here not only further establish allosteric inhibition as an alternative molecular therapy approach for the treatment of Ph+ ALL, but also evidence the need for an accurate definition of the breakpoint on der22 in order to optimize treatment. Furthermore there is the need for alternative allosteric inhibitors of the ABL-kinase, which overcome the partial resistance of $p185^{BCR/ABL}$ and the related "P-loop" mutants to allosteric inhibition.

Collectively our data show a difference between $p185^{BCR/ABL}$ and $p210^{BCR/ABL}$ with regard to their sensitivity towards allosteric inhibition, a difference which must be considered when using this novel approach for the treatment of Ph+ ALL.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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References

1. Radich JP. The biology of CML blast crisis. Hematology Am Soc Hematol Educ Program. 2007:384-91.
2. Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R, Kantarjian HM. The biology of chronic myeloid leukemia. N Engl J Med. 1999;341(3):164-72.
3. Melo JV, Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. Nat Rev Cancer. 2007;7 (6):441-53.
4. Ottmann OG, Wassmann B. Treatment of Philadelphia chromosome-positive acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2005:118-22.
5. Harnois T, Constantin B, Rioux A, Grenioux E, Kitzis A, Bourmeyster N. Differential interaction and activation of Rho family GTPases by p210bcr-abl and p190bcr-abl. Oncogene. 2003;22(41):6445-54.
6. Gokbuget N, Hoelzer D. Treatment of adult acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2006:133-41.
7. Zheng X, Oancea C, Henschler R, Moore MA, Ruthardt M. Reciprocal t(9;22) ABL/BCR fusion proteins: leukemogenic potential and effects on B cell commitment. PLoS One. 2009;4(10):e7661.
8. Li S, Ilaria RL Jr, Million RP, Daley GQ, Van Etten RA. The P190, P210, and P230 forms of the BCR/ABL oncogene induce a similar chronic myeloid leukemia-like syndrome in mice but have different lymphoid leukemogenic activity. J Exp Med. 1999;189(9): 1399-412.
9. Ramirez P, DiPersio JF. Therapy options in imatinib failures. Oncologist. 2008;13(4): 424-34.
10. Hantschel O, Superti-Furga G. Regulation of the c-Abl and Bcr-Abl tyrosine kinases. Nat Rev Mol Cell Biol. 2004;5(1):33-44.
11. Adrian FJ, Ding Q, Sim T, Velentza A, Sloan C, Liu Y, et al. Allosteric inhibitors of Bcr-abl-dependent cell proliferation. Nat Chem Biol. 2006;2(2):95-102.
12. Beissert T, Hundertmark A, Kaburova V, Travaglini L, Mian AA, Nervi C, et al. Targeting of the N-terminal coiled coil oligomerization interface by a helix-2 peptide inhibits unmutated and imatinib-resistant BCR/ABL. Int J Cancer. 2008;122(12): 2744-52.
13. Nijmeijer BA, Szuhai K, Goselink HM, van Schie ML, van der Burg M, de Jong D, et al. Long-term culture of primary human lymphoblastic leukemia cells in the absence of serum or hematopoietic growth factors. Exp Hematol. 2009;37(3):376-85.
14. Zhang J, Adrian FJ, Jahnke W, Cowan-Jacob SW, Li AG, Iacob RE, et al. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. Nature. 2010;463(7280): 501-6.
15. Mian AA, Oancea C, Zhao Z, Ottmann OG, Ruthardt M. Oligomerization inhibition, combined with allosteric inhibition, abrogates the transformation potential of T315I-positive BCR/ABL. Leukemia. 2009; 23(12):2242-7.
16. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science. 1990;247(4946):1079-82.
17. Ilaria RL Jr, Van Etten RA. P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. J Biol Chem. 1996;271(49):31704-10.