Small interfering RNA against *BCR-ABL* transcripts sensitize mutated T315I cells to nilotinib

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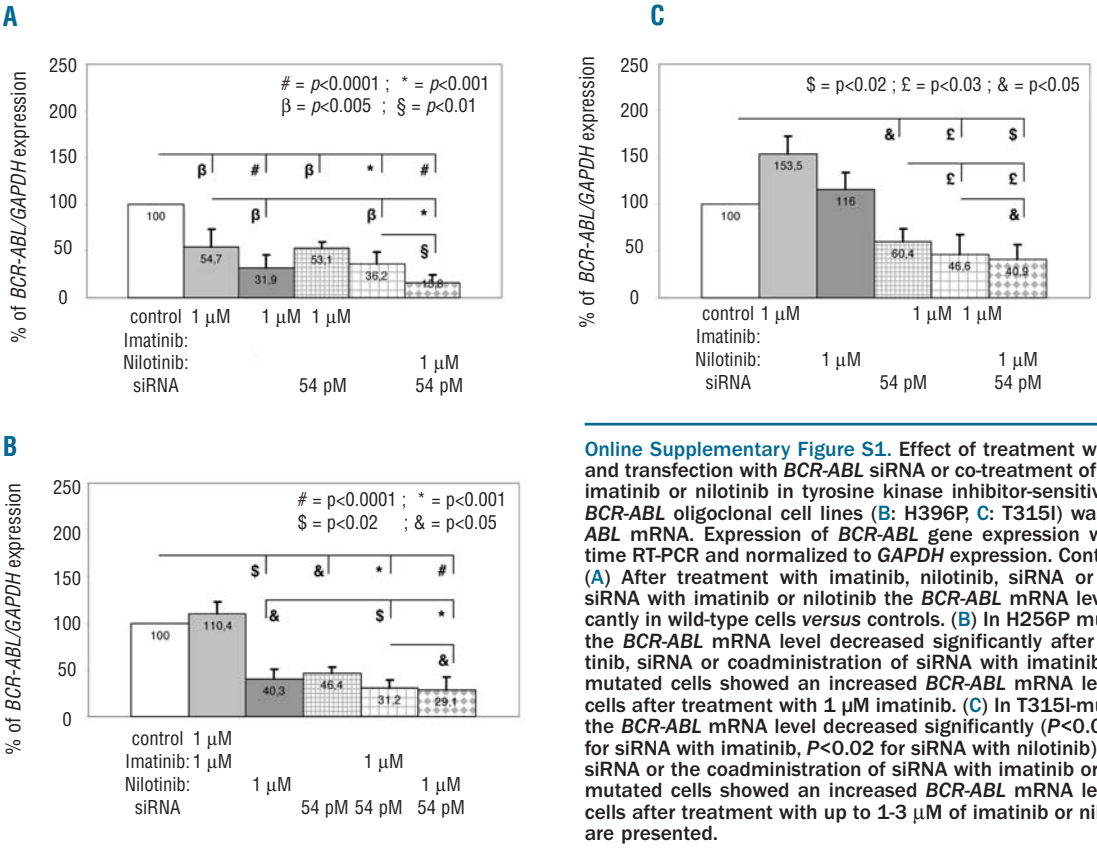
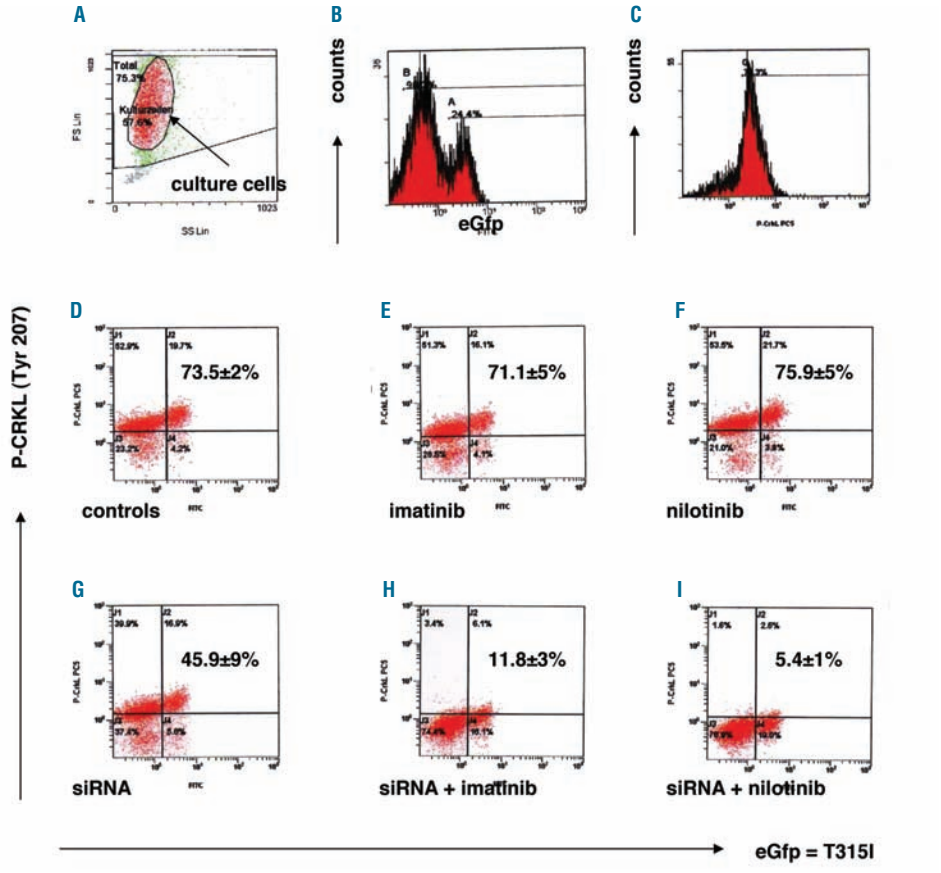
Online Supplementary Table S1. Effects of *BCR-ABL* siRNA on cell growth rate and on the *BCR-ABL* mRNA level of six patients with imatinib-resistant leukemic cells.

Diagnosis	stage	BCR-ABL mutations	*BCR-ABL* mRNA level in peripheral blood	Cell growth in % after transfection with *BCR-ABL* siRNA, controls were set up at 100%	*P* value	*BCR-ABL* gene expression in % after transfection with BCR-ABL siRNA, controls were set up at 100%	*P* value
CML	BC	Y253F	6.6	39.7 ± 14.3	<0.001	67.7 ± 9.1	<0.05
CML	BC	not done	16.4	46.1 ± 12.9	<0.001	57.7 ± 10.9	<0.01
CML	CP	F317L	0.6	67.5 ± 6.4	<0.01	78.9 ± 8.4	<0.05
CML	CP	E255K	0.7	57.4 ± 10.7	<0.01	65.6 ± 10.7	<0.05
AML	relaps	not done	1.14	20.1 ± 5.9	<0.0001	18.4 ± 6.0	<0.0001
ALL	relaps	not done	4.22	50.9 ± 9.5	<0.001	48.5 ± 18.5	<0.006

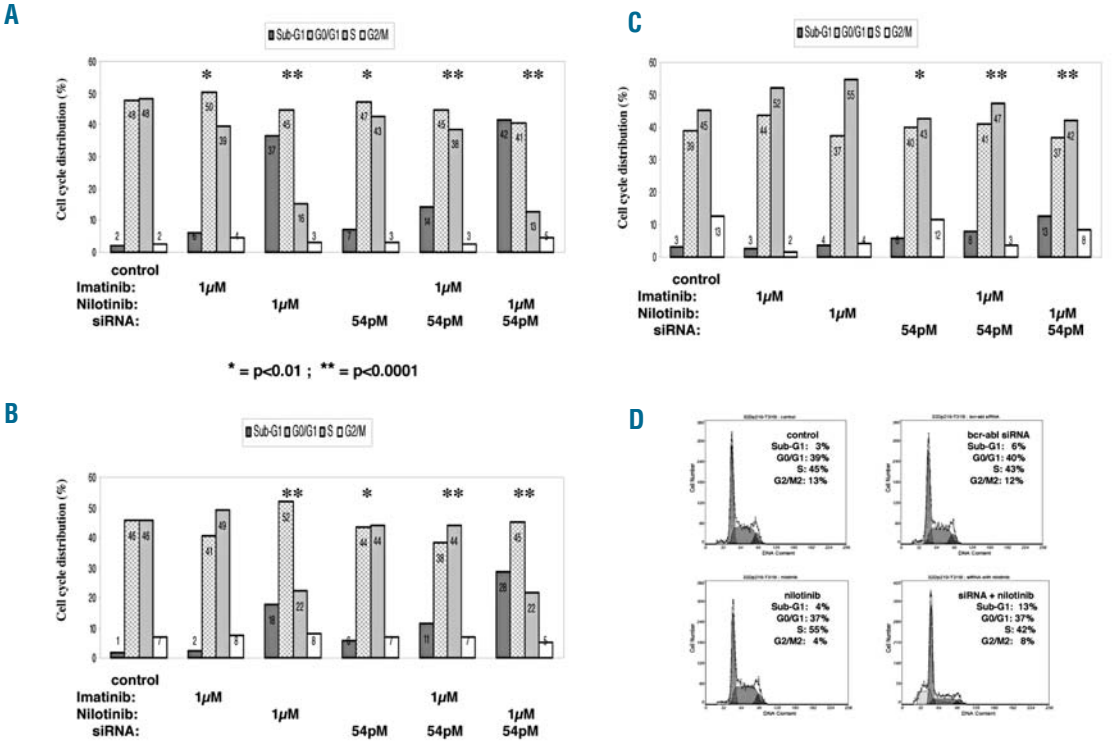
Cell growth was measured in triplicate by MTT assay and results are shown as mean ± SD. BCR-ABL *gene expression was measured in quintuplicate by real time RTPCR and results are shown as mean+SD normalized to* GAPDH *or 6-glucosephosphatdehydrogenase gene expression. Controls were set at 100%. Diagnosis and clinical stage are presented; BC: blast crisis; CP: chronic phase; BCR-ABL mutations according to the mutation of the kinase domain. Peripheral blood* BCR-ABL *mRNA levels are shown according to the International Scale.*



Online Supplementary Figure S1. Effect of treatment with imatinib, nilotinib and transfection with *BCR-ABL* siRNA or co-treatment of *BCR-ABL* siRNA with imatinib or nilotinib in tyrosine kinase inhibitor-sensitive (A) and –resistant *BCR-ABL* oligoclonal cell lines (B: H396P, C: T315I) was measured by *BCR-ABL* mRNA. Expression of *BCR-ABL* gene expression was detected by real time RT-PCR and normalized to *GAPDH* expression. Control was set at 100%. (A) After treatment with imatinib, nilotinib, siRNA or coadministration of siRNA with imatinib or nilotinib the *BCR-ABL* mRNA level decreased significantly in wild-type cells *versus* controls. (B) In H256P mutated BCR-ABL cells the *BCR-ABL* mRNA level decreased significantly after treatment with nilotinib, siRNA or coadministration of siRNA with imatinib or nilotinib. H396P mutated cells showed an increased *BCR-ABL* mRNA level *versus* untreated cells after treatment with 1 µM imatinib. (C) In T315I-mutated BCR-ABL cells the *BCR-ABL* mRNA level decreased significantly ($P<0.05$ for siRNA, $P<0.03$ for siRNA with imatinib, $P<0.02$ for siRNA with nilotinib) after treatment with siRNA or the coadministration of siRNA with imatinib or nilotinib. The T315I-mutated cells showed an increased *BCR-ABL* mRNA level *versus* untreated cells after treatment with up to 1-3 µM of imatinib or nilotinib. Mean and SD are presented.



Online Supplementary Figure S2. Representative flow cytometry profiles comparing phosphor-Crkl (p-Tyr207) populations during treatment with imatinib, nilotinib and transfection with *BCR-ABL* siRNA or cotreatment of *BCR-ABL* siRNA with imatinib or nilotinib in T315I-resistant *BCR-ABL* oligoclonal cell lines. (A) Representative flow cytometry FSC/SSC density plot gated on 32Dp210-Thr315Ile cells. (B) The frequency of the T315I mutated BCR-ABL population expressed as a percentage of all 32Dp210 cells. (C) The frequency of the p-Crkl population expressed as a percentage of all 32Dp210-Thr315Ile cells. (D) Representative flow cytometry dot plot showing the expression of p-Crkl after treatment with non-silencing siRNA (mismatched or scrambled siRNA). (E-I) Representative flow cytometry dot plots showing the expression of p-Crkl after treatment with imatinib, nilotinib, *BCR-ABL* siRNA or the coadministration of siRNA with imatinib or nilotinib. After treatment with siRNA, coadministration of siRNA with imatinib or nilotinib the phosphor-Crkl protein (p-Tyr207) level decreased significantly in T314I-mutated BCR-ABL cells *versus* controls.



Online Supplementary Figure S3. Effects of treatment with imatinib, nilotinib and transfection with *BCR-ABL* siRNA or cotreatment of *BCR-ABL* siRNA with imatinib or nilotinib in tyrosine kinase inhibitor-sensitive (A) and –resistant *BCR-ABL* oligoclonal cell lines (B: H396P, C: T315I) on the cell cycle. (A) After treatment with nilotinib and coadministration of siRNA with imatinib or nilotinib the sub-G1 value increased significantly to up to 41.9% (36.8% for nilotinib, 14.4% for siRNA with imatinib, and 41.9% for siRNA with nilotinib) in wild-type cells *versus* controls (mean 2.0%). Concordantly the S-phase decreased significantly to 12.9% (15.5% for nilotinib, 38.4% for siRNA with imatinib, and 12.9% for siRNA with nilotinib) in wild-type cells *versus* controls (mean 48.1%). The G0/G1-phase and the G2/M-phase showed moderately changed cell-cycle distribution in wild-type cells. (B) In H396P mutated BCR-ABL cells the strongest effects were seen with nilotinib and siRNA with nilotinib affecting induction of the sub-G1 value and reduction of the S-phase. (C) In the T315I-mutated BCR-ABL cells the strongest induction of the sub-G1 value was seen after treatment with siRNA, siRNA with imatinib, and siRNA with nilotinib *versus* controls. S-phase inhibition was achieved with siRNA and with the combination of nilotinib with siRNA compared to controls. The profiles of DNA contents (measured by flow cytometry) are presented as bars indicating cell-cycle distribution and represent the mean of three independent experiments. (D) Representative profiles of DNA content and the cell-cycle distribution using MultiCycle™ software after treatment with untreated control cells or non-silencing siRNA, nilotinib, *BCR-ABL* siRNA or coadministration of siRNA with nilotinib in the 32Dp210-Thr315Ile cells.