Simultaneous MEK/ERK and BCR-ABL inhibition partially restores cell death induced by the tyrosine kinase inhibitor imatinib

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Deregulation of MEK/ERK signaling pathway has emerged as a BCR-ABL-independent mechanism of imatinib (IM) resistance in chronic myeloid leukemia. In order to address this question, a cell line derived by exposition of K562 cell line to increasing concentrations of IM was developed. The K-IM cell line exhibited no BCR-ABL mutation, no efflux transporter activity, nor alteration on XIAP levels. On the other way, activation of MAPK/ERK signaling and survivin overexpression were shown to be important mechanisms of IM resistance in K-IM cell line. Increasing in IM concentration overcame resistance mediated by BCR-ABL amplification and survivin, but the K-IM cell line remained significantly more resistant than the parental cell line. This result suggests a role for BCR-ABL in the resistance of the cell line. Isolated inhibition of MEK/ERK had no effect on cell death. However, MEK/ERK and BCR-ABL inhibition potentiated the IM-induced cell death. The K-IM cell line is an intriguing model for the study BCR-ABL-dependent and -independent mechanisms of resistance. Our data reinforce the hypothesis of multifactorial resistance in cancer and points out to need of identification of molecular alterations that together could play a role in drug resistance.

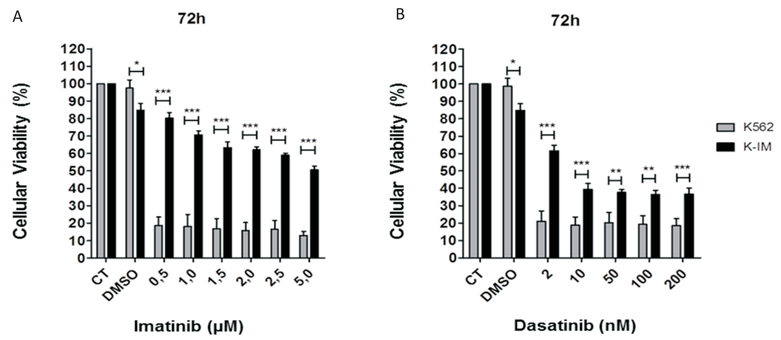


Figure 1: Cellular response to tyrosine kinase inhibitors. K562 and K-IM cell lines were incubated with different concentrations of imatinib (0.5 to 5.0 μM) (A) or dasatinib (2 to 200 nM) (B) for 72 hours and cell viability was assessed using the MTT assay. Data corresponds to 3 independent experiments bars represent standard error. Statistical analysis was performed using the two-way ANOVA test with Bonferroni post-test in which * corresponds to p<0,05; ** corresponds to p<0,01 and *** corresponds to p<0,001.

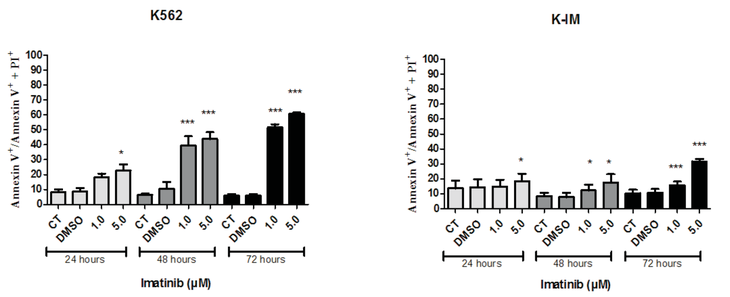


Figure 2: Cell death induction by imatinib. K562 and K-IM cell lines were treated with 1 or 5 μM imatinib or the vehicle DMSO for 24, 48 or 72 hours. Cell death induction was assessed by annexin V and propidium iodide. Statistical analysis was performed using the one-way ANOVA test with Dunnett post-test in which * corresponds to p<0,05 and *** corresponds to p<0,001.

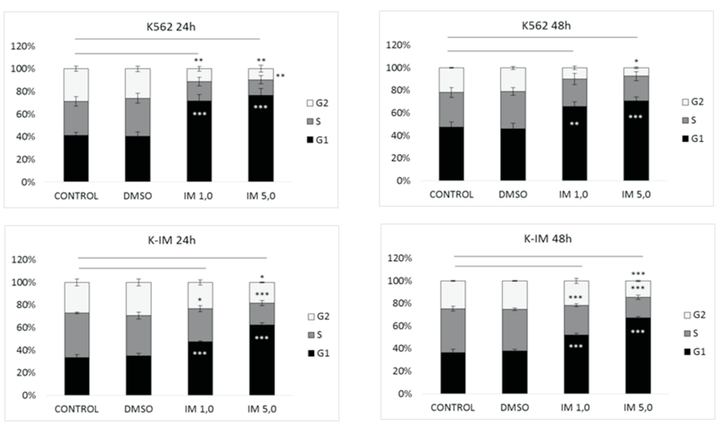


Figure 3: Cell cycle analysis after imatinib treatment. K562 and K-IM cell lines were treated with 1 or 5 μM imatinib (IM) or the vehicle DMSO for 24 or 48 hours. Percentages of cells in each cell cycle phase is shown in the graphs.

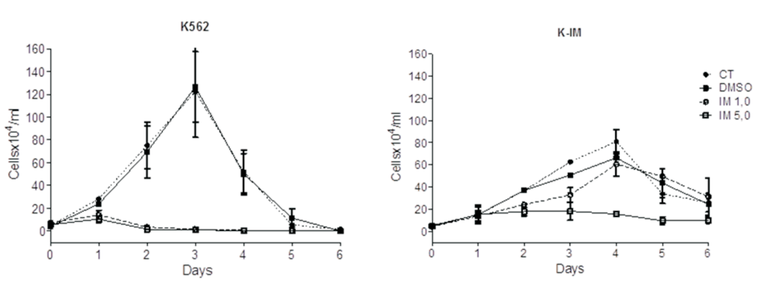
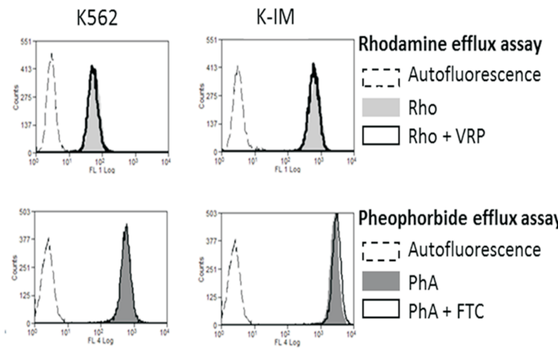


Figure 4: Cell growth evaluation. K562 (A) and K-IM (B) cells were treated with 1 or 5 μM imatinib (IM) or the vehicle DMSO and cells were counted using the tripan the tripean blue exclusion method every 24 hours for 6 days. Data corresponds to two independent experiments and bars represente standard deviation.



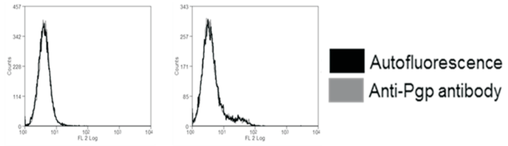


Figure 5: Evaluation of efflux transport proteins. P-glycoprotein (Pgp) and breast cancer resistance protein (BCRP) activity was evaluated. Cells were incubated with the fluorescent substrate Rhodamine 123 (Rho) in the presence or absence of the Pgp modulator verapamil (VRP) and analized in the flow cytometer for Pgp activity. Cells were incubated with the fluorescent substrate Pheophorbide A (PhA) in the presence or absence of the BCRP modulator Fumitremorgin C (FTC) and analized in the flow cytometer for BCRP activity. B) K562 and K-IM cells were incubated with anti-Pgp antibody conjugated to ficoeritrin and analyzed in the flow cytometer.

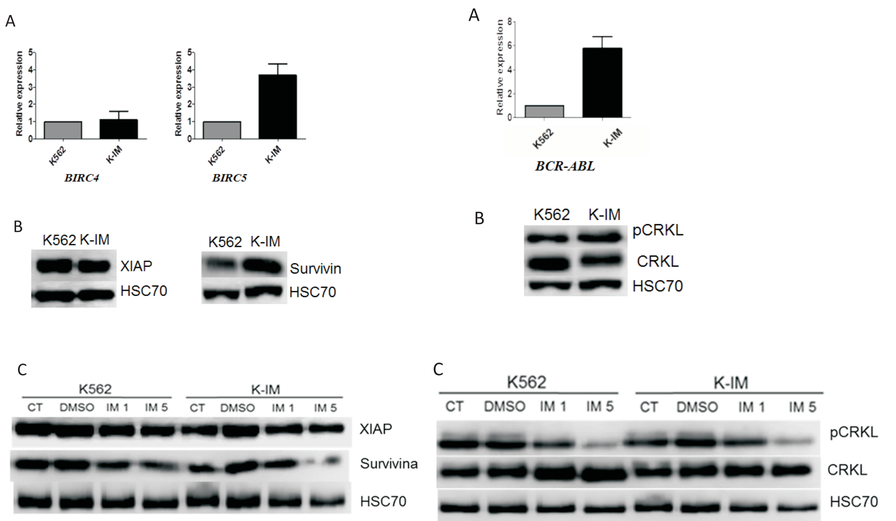


Figure 6: Apoptosis inhibitor proteins. A) *BIRC 4* (XIAP) and *BIRC 5* (survivin) mRNA levels were assessed using RT-qPCR. β-glucuronidase (*GUSB*) gene was used as endogenous control. B) XIAP and survivin protein levels were assessed by western blot and HSC70 protein was used as control. C) K562 and K-IM cell lines were treated with 1 or 5 μM imatinib (IM) or the vehicle DMSO for 24 hours and survivin protein levels were assessed using western blot. HSC70 protein was used as control.

Figure 7: Evaluation of BCR-ABL status: A) mRNA levels were analyzed using RT-qPCR in the K-IM cell line relative to K562 cell line. β-glucuronidase (GUSB) gene was used as endogenous control. B) BCR-ABL activity was indirectly evaluated through the CRKL phosphorylation levels, total CRKL and HSC70 protein levels were evaluated as control. C) For the assessment of BCR-ABL inhibition by imatinib (IM) treatment, K562 and K-IM cells were treated with 1 or 5 μM of IM or the vehicle DMSO for 24 hours. The protein content and phosphorylation levels of CRKL protein were assessed using Western Blot. HSC70 protein was used as sample loading control.

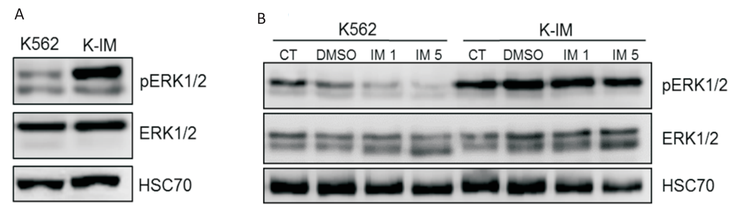


Figure 8: ERK1/2 proteins evaluation. A) ERK1/2 proteins total and phosphorylated levels were evaluated in K562 and K-IM cell lines. B) K562 and K-IM cell lines were treated with 1 or 5 μM imatinib (IM) or the vehicle DMSO for 24 hours and ERK1/2 proteins total and phosphorylated levels were assessed using western blot. HSC70 protein was used as loading protein control.

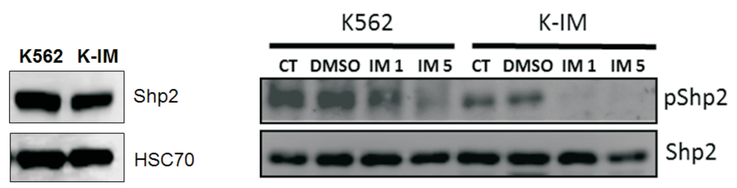


Figure 9: Shp2 protein evaluation. A) Shp2 total and phosphorylated protein basal levels were evaluated in K562 and K-IM cell lines. B) K562 and K-IM cell lines were treated with 1 or 5 μM (IM) or the vehicle DMSO for 24 hours and Shp2 protein total and phosphorylated levels were assessed using western blot.

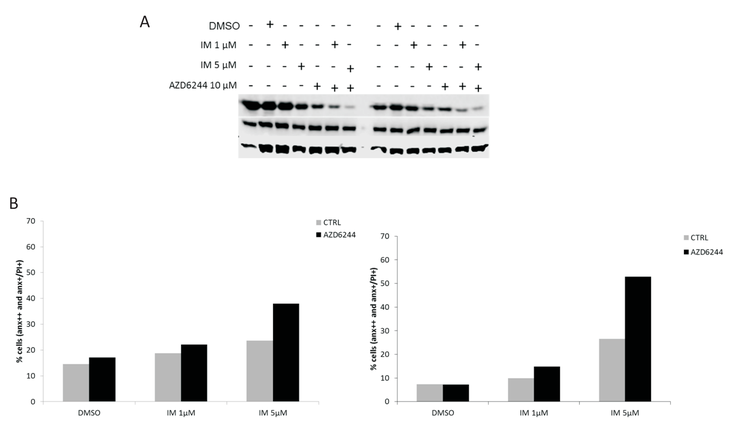


Figure 10: MEK1/2 inhibition and cell death induction in K-IM cell line. A) K-IM cell line was treated with 1 μM and 5 μM imatinib (IM), 10 μM AZD6244 (AZD) or the vehicle DMSO for 24 and 48 hours and ERK1/2 protein total and phosphorylated levels were assessed using western blot . HSC70 protein was used as loading protein control. B) Cell death was analyzed in the same conditions as in A.

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