

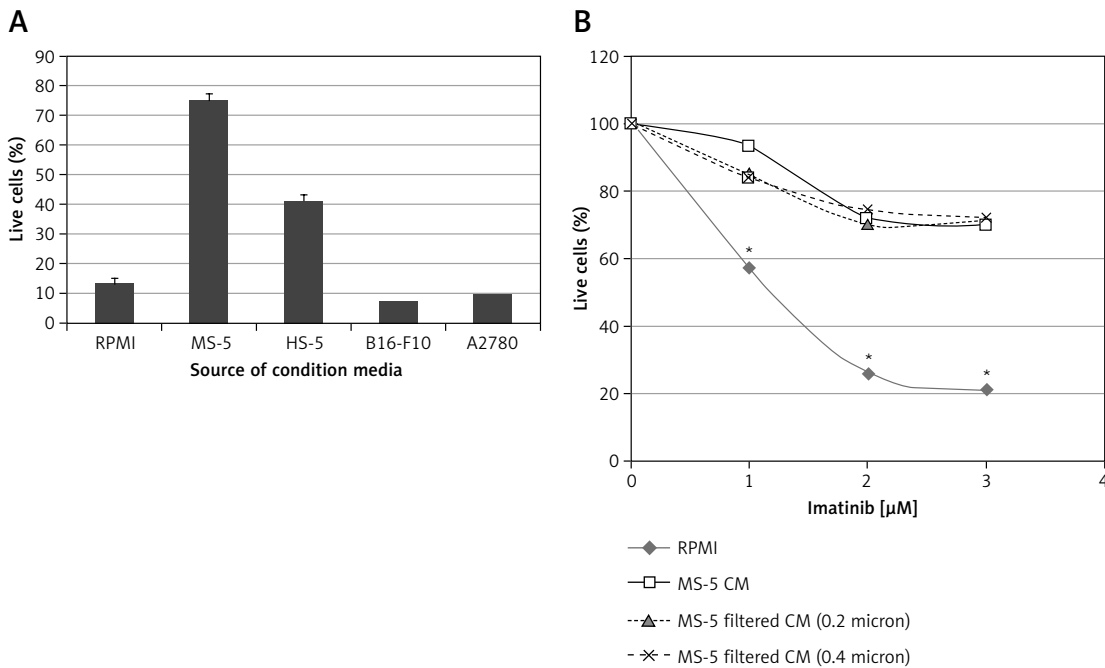
Supplemental data

Mesenchymal soluble factors confer imatinib drug resistance in chronic myelogenous leukemia cells

To address whether conditioned media (CM) collected from other cell lines might promote resistance to imatinib in MEG-01 cells, CM were collected from A2780, an ovarian cancer cell line, murine mesenchymal stem cells (MS-5), human mesenchymal stem cells (HS-5) and murine skin melanoma cells (B16-F10). MEG-01 cells were exposed to the different CM in the presence of 1 μ M imatinib. Results in Supplementary Figure S1 A show that A2780 and B16-F10 CM were not efficient at conferring drug resistance to imatinib.

To determine the nature of the active component in the CM that mediates drug resistance to

imatinib in CML cells, we investigated whether the activity of MS-5 CM is mediated by microvessels or soluble factors released from the mesenchymal stem cells to the growth medium. CM was collected from MS-5 cells and passed through 0.4- and 0.2- μ m filters, and the ability of the filtered CM to induce imatinib drug resistance was investigated. There was no significant change in activity between the CM and either filtered CM (Supplementary Figure S1 B), suggesting that CM activity is due to components that are smaller than 0.2 μ m in size, and ruling out the potential involvement of microvessels in mediating this phenomenon.



Supplementary Figure S1. Selectivity and initial characterization of mesenchymal stem cell conditioned medium. **A** – Percentage of live (remaining) cells; measured by trypan blue assay, of MEG-01 cells treated with 1 μ M imatinib in the presence of conditioned medium (CM) collected from MS-5, HS-5, B16-F10 and A2780 cultures. Negative control included fresh RPMI medium. **B** – Percentage of live cells, measured by trypan blue assay, of MEG-01 cells exposed to increasing concentrations of imatinib in the presence of MS-5 CM or filtered CM (0.2 or 0.4 μ m). *Significantly different from untreated cells at $p < 0.01$. The experiment was repeated twice, with a comparable outcome