Abstract. Imatinib mesylate (STI571), an inhibitor of alpha- and beta-platelet-derived growth factor receptors (PDGFR) and other tyrosine kinases, is a well established treatment for chronic myeloid leukaemia and gastrointestinal stromal tumours. Moreover, it is under investigation for the therapy of several other malignant tumours since protein kinases are frequently mutated or otherwise deregulated in human malignancies and they serve as a target for differentiating between tumour cells and normal tissues. The objective of this study was to determine whether gamma radiation could sensitize astrocytoma cell lines to the effects of imatinib in vitro. For this purpose, T98G and MOG-G-UVW astrocytoma cells were treated with imatinib alone or in combination with gamma radiation. The clonogenic survival assays performed with the combination of imatinib with radiation demonstrated that the drug had an additive antiproliferative effect in both cell lines considered. Imatinib conferred greater radiosensitivity on the T98G tumour cells effecting a significant decrease in colony formation compared with radiation alone. These data provide a rationale to further investigate the combination of imatinib with radiation, keeping in mind that this may result in unexpected toxicities that are not observed with either treatment alone.

Imatinib mesylate (formerly STI571) is a 2-phenylamino-pyrimidine compound that selectively inhibits the catalytic action of the c-Abl kinase, stem cell factor receptor (c-Kit) and platelet-derived growth factor receptor (PDGFR) tyrosine kinases. It is approved for the treatment of chronic myeloid leukaemia and gastrointestinal stromal tumours where there is constitutive activation of one of its kinase targets (1, 2). Moreover, its efficacy is currently under investigation for several other neoplasms including malignant brain tumours (i.e. astrocytoma), lung cancer and other solid tumors (3-5).

Astrocytoma is a brain tumour characterized by several alterations of the PDGF/PDGFR signal transduction pathway, including protein overexpression and autocrine and paracrine ligand stimulation (6), suggesting that autocrine PDGFR stimulation may contribute to its growth. The disruption of this loop by neutralizing anti-PDGF antibodies or dominant-negative mutants of PDGF and PDGFR has led to growth inhibition and reversion of the transformed phenotype in cell lines (7), suggesting that PDGFR may be a target for the therapy of malignant astrocytoma (8). Today malignant astrocytoma treatment is mainly based on a combination of surgery, radiation therapy and chemotherapy, however, despite these treatment modalities, the responses are extremely poor (9, 10).

Since radiotherapy is an important treatment option in many tumours, particularly for astrocytoma, the combined effects of imatinib and radiation were analyzed in this study in two human malignant astrocytoma cell lines. Indeed, similar studies performed in other tumour cell lines, such as epidermoid carcinoma and neuroblastoma (11, 12), have demonstrated that imatinib associated with fractionated radiotherapy can reduce tumour growth.

Materials and Methods

Cells. Human glioblastoma T98G cells and human anaplastic astrocytoma MOG-G-UVW cells were purchased from the European Collection of Cell Culture (ECACC, Salisbury, UK) and cultured in RPMI-1640 and DMEM/HAM’S F10 medium respectively, supplemented with 10% heat-inactivated foetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin and 100 μg/ml streptomycin. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Drug. Imatinib mesylate was kindly provided by Dr E. Alessandrino; a 17 mM stock solution was prepared in distilled sterile water and stored at −20°C. In this study imatinib was used at increasing concentrations from 1 to 30 μM.
Gamma irradiation. Cells were irradiated with 0, 0.25, 2 or 5 Gy using a CGR Alcyon II (Fondazione IRCCS, Policlinico S. Matteo, Pavia, Italy) with a cobalt-60 source at a dose rate of 1 Gy/min.

Clonogenic assay. The effects of imatinib alone or in combination with γ-rays were tested by means of clonogenic survival assay. Briefly, the cells were plated in triplicate at different densities (1×10^2; 1.7×10^2; 2.7×10^2) according to the dose and precultured for 24 h, then treated with imatinib (0, 1, 5, 10 μM) for 1 h prior to gamma irradiation. Irradiated cells were cultured in medium supplemented with imatinib for 12 days, renewing the complete medium with imatinib 6 days after irradiation. Colonies were stained with crystal violet and scored as survivors when constituted by more than 50 cells. The surviving fractions were calculated relative to the mean plating efficiency of unirradiated flasks of the same experiment. Statistical Analysis. Comparisons between groups were made by the students' t-test. A value of p<0.05 was considered significant.

Results and Discussion

Effects of imatinib on clonogenic survival. In the first group of experiments the clonogenic survival of the T98G and MOG-G-UVW cells after incubation with different concentrations of imatinib mesylate alone was evaluated. The results, summarized in Figure 1A, showed that therapeutic concentrations (1-10 μM) of the drug were not able to effect significant differences in clonogenic activity compared to control conditions in both cell lines. Higher concentrations (i.e. 20 and 30 μM) were sufficient to induce a significant (p<0.01) decrease in colony formation. Subsequently, 12.5 μM, 15 μM and 17.5 μM were also tested, to better investigate the concentration dependence of colony formation in these cell lines. A concentration of 12.5 μM was able to halve the clonogenic capacity of the T98G cells (Figure 1B) and to almost nullify that of the MOG-G-UVW cells. However, low concentrations (i.e. 5 and 10 μM) of imatinib were observed to induce a decrease of colony expansion and scattering (Figure 2) compared to control conditions, although without any difference in the clonogenic survival fraction. This morphologically observable effect may be explained by partial arrest of the cell growth. Indeed, we recently found that imatinib was able to induce growth arrest in the G₀/G₁ phase of the cell cycle in astrocytoma cells, suggesting a possible cytostatic effect of this new drug (13).

Effects of imatinib in combination with radiation on clonogenic survival. On the basis of the previous experiments, the effects of low-dose imatinib (1, 5 or 10 μM) after combination with gamma irradiation on the clonogenic survival of the T98G and MOG-G-UVW cells was investigated. With the T98G cells the effect of radiation (2
Gy dose) was increased by imatinib at the clinically tolerable concentration of 5 μM. This effect was even more evident when gamma irradiation was associated with an imatinib dose of 10 μM. Therefore, imatinib had an additive antiproliferative effect on the T98G cells compared to irradiation alone. Comparison of the survival data of the T98G cells and MOG-G-UVW cells showed that the two cell lines behave differently with the combined treatment, since no significant synergism of the drug with radiation was found in the MOG-G-UVW cells (Figure 3).

**Conclusion**

Imatinib is able to effect a significant decrease *in vitro* in clonogenic activity of astrocytoma cells, and moreover acts in a synergistic manner with gamma radiation on the reduction of clonogenic survival of T98G glioblastoma cells. Clinical doses (1-10 μM) of imatinib mesylate enhance the radiosensitivity of T98G cells, but not of MOG-G-UVW with T98G cells the reduction in clonogenic survival with the combined treatment is comparable to that with radiation alone.

Further studies are warranted to evaluated the effects of imatinib on other astrocytoma cells to evaluate its clinical development as a radiosensitizer in the treatment of patients with malignant astrocytoma and to better understand the molecular mechanisms underlying the synergic response to the combined treatments. Indeed, the pathways downstream of PDGFR in this response are as yet unknown, but signal transducer and activator of trascriptation protein (STAT), which is activated by PDGFR and implicated in radioresistance in Bcr-Abl-expressing cells, is one of the possible candidates (8).

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**References**


**Figure 3.** Clonogenic survival of T98G (A) and MOG-G-UVW (B) cells exposed to 0, 0.25 or 2 Gy with different doses of imatinib. S.F., surviving fraction.

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