Table 2: Number of cells counted per control membrane, and percent invasion (mean ± standard deviation) of cells in response to experimental conditions.

Cell Line	Average Number of Cells in Control PET	Percent Invasion of Cells in Response to 10% FBS	Percent Invasion of Cells in Response to Imatinib mesylate
MKT-BR	247	38.43% ± 8.5	1.03% ± 0.2
OCM-1	246.67	21.7% ± 1.9	0.1% ± 0.2
92.1	223	14.4% ± 2.7	0.2% ± 0.2
UW-1	66.33	12% ± 4.8	0% ± 0
SP6.5	266	3% ± 1.5	0% ± 0

Results

Fifty-five cases of UM were studied. Eight seven percent of the tumors (n = 48) were classified as mixed cell type (spindle and epithelioid cells), 9% (n = 5) had predominance of epithelioid cells, and 3.6% (n = 2) of spindle cells.

Seventy-eight percent of cases (n = 43) were found to be c-kit positive (Figure 1A). Among the positive cases, 46.5% (n = 20) presented with what was considered as high expression. All lesions with high immunoreactivity (n = 20) had cytoplasmic and cell membrane expression. Meanwhile, among lesions with low immunoreactivity (n = 23), 100% presented a cytoplasmatic reaction and just 30.4% (n = 7) presented with a cell membrane stain-pattern (Figure 1B). (Table 1)

The percent invasion of cell lines according to the baseline invasion without imatinib mesylate was: MKT-BR (38.4%) > OCM-1 (21.7%) > 92.1 (14.4%) > UW-1 (12%) > SP6.5 (3%). The addition of imatinib mesylate decreased the invasion in all cell lines: MKT-BR (1.03%); OCM-1 (0.1%); 92.1 (0.2%); UW-1 (0%); SP6.5 (0%). The results are shown in Figure 3.

No visible changes in cytomorphology were seen in reaction to the presence of imatinib mesylate (figure 2).

Statistically significant differences between the invasion rates for the control group and imatinib mesylate group were found in all cell lines (T test p value < 0.05).

Figure 4 depicts the results from the Sulforhodamine-B assay. The mean and standard deviation for each cell line per condition is shown in Table 2. Cells that were directly exposed imatinib mesylate showed a decrease in proliferation for all five human cell lines (92.1, MKT-BR, OCM-1, SP6.5, UW-1) as compared to control (p value of 0.001354991, 0.012655861, 9.47698×10^{-7}, 0.002754018 and 5.79576×10^{-6} respectively).

Discussion

It is known that protein tyrosine kinases (PTK) have an important role in cellular mechanisms, such as differentiation, proliferation and regulatory mechanisms, as well as in signal transduction. C-kit is one of these PTK, which is expressed in a wide variety of human malignancies [16] including chronic and acute myelogenous leukemia [6], GIST [7], mastocytosis [17], small cell lung carcinoma [18], chromophobe renal cell carcinoma [19], cutaneous [20] and UM [16]. As c-kit is expressed in normal interstitial cells of Cajal, the progenitor cell of GIST [7], the present article studies the expression of c-kit in uveal melanomas, as normal choroidal melanocytes do express this marker [21].

We demonstrated that more than 75% of UM from our series are positive for c-kit. This finding, per se, supports the idea of a clinical trial of imatinib mesylate for UM, especially in metastatic cases. Once metastatic disease is detected, no effective method of systemic therapy has been identified [3]. Moreover, not 100% of GIST is positive for c-kit. In fact, 6% of GIST are c-kit negative [22]. Before the imatinib mesylate "era", metastatic GIST had a median survival times ranging between 10–20 months [23]. Nowadays, imatinib mesylate controls tumor growth in up to 85% of advanced GIST [24], with 90% of acceptable toxicity [25].

In cutaneous melanoma, c-kit is strongly expressed in radial growth phase, and weak or no expression is seen in vertical growth phase and metastatic disease [26]. Therefore, in cutaneous melanomas c-kit expression appears to be related with stage of the disease. To further investigate a similar expression of the c-kit in UM, we observed the cell type (spindle and epithelioid) in which the c-kit was expressed, as it is well known that spindle cell type is less aggressive than epithelioid type [1]. None of the previous studies recorded the cell type in which the expression was occurring. Mouriaux *et al* [21] compared the c-kit expression with cell type tumor (Callendar's classification) as a correlation to prognostic factor, but did