SUMMARY

This study compared for the first time the *in vitro* effects of different bisphosphonates on melanoma cell lines. Three compounds with different mechanism of action and different antiresorptive potency were analysed: a non-amino-bisphosphonate (clodronate) and two amino-bisphosphonates, pamidronate and zoledronate, which is the most potent antiresorptive agent known to date. Their effects on cell proliferation, induction of apoptosis and cell cycle progression in the melanoma cell lines A375 and M186 and in the Bcl-2 overexpressing cell line A375/ Bcl-2 have been investigated in detail.

In this experimental model, it could be shown that the nitrogen-containing bisphosphonates inhibit cell proliferation, induce apoptosis and alter cell cycle progression causing accumulation of cells in the S phase of the cycle. These effects were dose dependent, and were achieved at concentrations ranging from 10 to 100 μM. The antiproliferative and proapoptotic activity of the two compounds *in vitro* did not correlate well with their known antiresorptive potency *in vivo*. However, despite a stronger effect of pamidronate in inducing apoptosis, the more potent antiresorptive zoledronate was more effective in inhibiting cell proliferation and altering cell cycle progression, showing a stronger influence on the overall tumor growth.

Apoptosis induced by the amino-bisphosphonates was dose and time dependent and acute exposure to pamidronate over 6h was sufficient to induce apoptosis. The pro-apoptotic effect of pamidronate and zoledronate was not inhibited by overexpression of Bcl-2 protein and did not appear to involve of cytochrome c release from the mitochondria, suggesting that these compounds may stimulate a mitochondria-independent pathway for inducing apoptotosis.

In contrast, the non-amino-bishophonate clodronate had no effect on cell proliferation, apoptosis induction or cell cycle progression in melanoma cell lines, even at high concentrations. This finding further confirms differences in the intracellular mechanism of action between the two types of bisphosphonates: the amino bisphosphonate (supposed to act by inhibition of protein prenylation) and the non-amino-bisphosphonates (who are reportedly metabolised to toxic ATP analogues). The latter mechanism seems to have no functional significance in melanoma cell growth.

In conclusion, amino-bisphosphonates manifest a direct antitumoral effect on melanoma cells *in vitro*, and may thus represent a promising novel class of agents for the treatment/prevention of melanoma metastasis. Further studies are required, in order to describe the exact mechanism of action of these compounds, their most effective structures and ultimately their potential place in new strategies for adjuvant therapy schedules in malignant melanoma.