The potential role of histone deacetylase inhibitors in the treatment of multiple myeloma

S. Deleu, K. Vanderkerken

This thesis contains in vitro and in vivo pre-clinical evaluations of a novel hydroxamate based histone deacetylase inhibitor, JNJ-26481585, developed by Johnson & Johnson. In the first part of this thesis we examined the anti-multiple myeloma (MM) activity of JNJ-26481585 as single agent. In vitro, we studied the downstream effects of JNJ-26481585 using the 5T33MMvt cell line. In vivo, we evaluated the potential value of JNJ-26481585 on the development of MM in two different settings namely in a prophylactic (5T33MM model) and in a therapeutic setting (5T2MM model). In the second part of this thesis, our aim was to investigate the effect of bortezomib (Velcade®) either as single agent or in combination therapy with JNJ-26481585 on the MM disease with special emphasis on the MM bone disease.
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Introduction

Multiple myeloma (MM) is a B-cell malignancy characterised by an accumulation of plasma cells in the bone marrow (BM) secreting monoclonal immunoglobulins. Reciprocal interactions between the tumour cells and the BM microenvironment result in increased cell survival, release of cytokines, development of drug resistance, angiogenesis and induction of bone disease. Despite recent improvements in the treatment of MM, MM remains an incurable disease. Anaemia, renal dysfunction and osteolytic bone disease are major and life-threatening clinical features of the disease. Therefore, the identification of new key targets in both the MM cells and the BM microenvironment is crucial for the development of new therapeutic strategies. Histone deacetylase inhibitors (HDACi) and bortezomib are drugs that target both malignant cells and the tumour microenvironment. HDACi represent a new class of anti-tumour agents. Inhibiting HDACs results in histone hyperacetylation and alterations in chromatine structure, which, in turn, cause growth arrest differentiation and/or apoptosis in several malignant cells. HDACi have anti-myeloma activity by directly inhibiting the proliferation of the malignant plasma cell (for instance by increasing the expression of pro-apoptotic genes and cell cycle inhibitors) but also by altering their environment, reducing secretion of growth factors (IL-6 and VEGF) and adhesion to bone marrow stromal cells.[1-2] Bortezomib is a proteasome inhibitor approved as first-line treatment of newly diagnosed MM patients. Pre-clinical and clinical data suggest that bortezomib also has a positive effect on bone remodelling by inhibiting osteoclast formation and stimulating osteoblast differentiation.[3]

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Key words: 5TMM model, bortezomib, combination therapy, HDAC inhibitors, MM bone disease, multiple myeloma

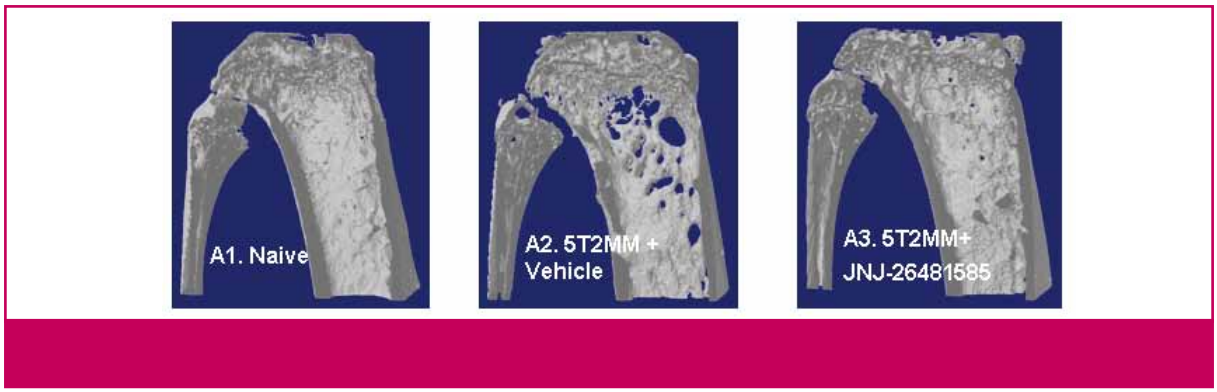

Figure 1. µCT images of the tibia from (A1) naïve, (A2) 5T2MM and (A3) 5T2MM-bearing mice treated with JNJ-26481585.

5TMM model

5TMM models are derived from elderly C57BL/KaLwRij strain that spontaneously developed myeloma. Several 5TMM lines were developed and have been propagated by intravenous (i.v.) transfer of the diseased marrow in young syngeneic, immunocompetent mice.[4] The 5T2 and 5T33MM are the most characterised and studied ones. Both models mimic the human disease closely, with the selective localisation of tumour cells in the BM, the presence of a serum M component, increased BM angiogenesis and induction of osteolytic bone disease (5T2MM).[5] Since the 5T33MM model has a rapid tumour growth, the 5T33MM model is often used in a prophylactic setting. In this setting treatment starts from the day after the i.v. injection of 5T33MM cells. The 5T2MM model has a more moderate growth and therefore we preferentially use this model in a therapeutic setting. Hereby, treatment starts once the MM disease is established in the BM, as determined by an increase of serum M spike, 8 weeks after i.v. injection of the 5T2MM cells.

Part I: effect of JNJ-26481585 as single agent in the development of MM

In vitro study

We first investigated the anti-MM activity of JNJ-26481585 in vitro using the 5T33MMvt cell line. FACS demonstrated that JNJ-26481585 induced apoptosis and cell cycle arrest at low nanomolar concentrations. These data were in line with the Western blot data demonstrating that JNJ-26481585 activated the intrinsic and extrinsic caspase cascade and upregulated p21 expression at low nanomolar concentrations, respectively. Similar results could be observed in normal BM endothelial cells using higher concentrations, indicating selectivity of JNJ-26481585 toward MM cells.[6]

In vivo study

The effect of JNJ-26481585 on tumour burden and angiogenesis in a prophylactic and therapeutic setting

Treatment of the 5T33MM mice with JNJ-26481585 (20 mg/kg of JNJ-26481585, every other day subcutaneous (s.c.)) resulted in a dramatic reduction of MM disease, as shown by a significant reduction of serum M component and BM plasmacytosis which was reduced for 85% compared with vehicle group ($p<0.05$). A similar effect could be observed in the 5T2MM model. Tumour burden in 5T2MM-bearing mice treated with JNJ-26481585 (20 mg/kg of JNJ-26481585, every other day s.c.) was reduced for more than 90% compared with vehicle group ($p<0.05$). In both prophylactic and therapeutic setting, treatment with JNJ-26481585 also decreased the newly formed blood vessels by 50 and 35% compared with vehicle group in the 5T33MM and 5T2MM model, respectively ($p<0.05$).[6]

The effect of JNJ-26481585 on the MM-related bone disease in the 5T2MM model

The development of the MM-related bone disease in 5T2MM-bearing mice (vehicle control group) was characterised by a significant increase of osteolytic lesions, a decrease in percentage trabecular bone volume, a decrease in trabecular number and an increase in the percentage osteoclasts. As shown in *Figure 1*, µCT images indicated that formation of the osteolytic lesions was dramatically reduced by 70% in mice treated with JNJ-26481585 ($p<0.05$). Furthermore, trabecular bone volume, trabecular

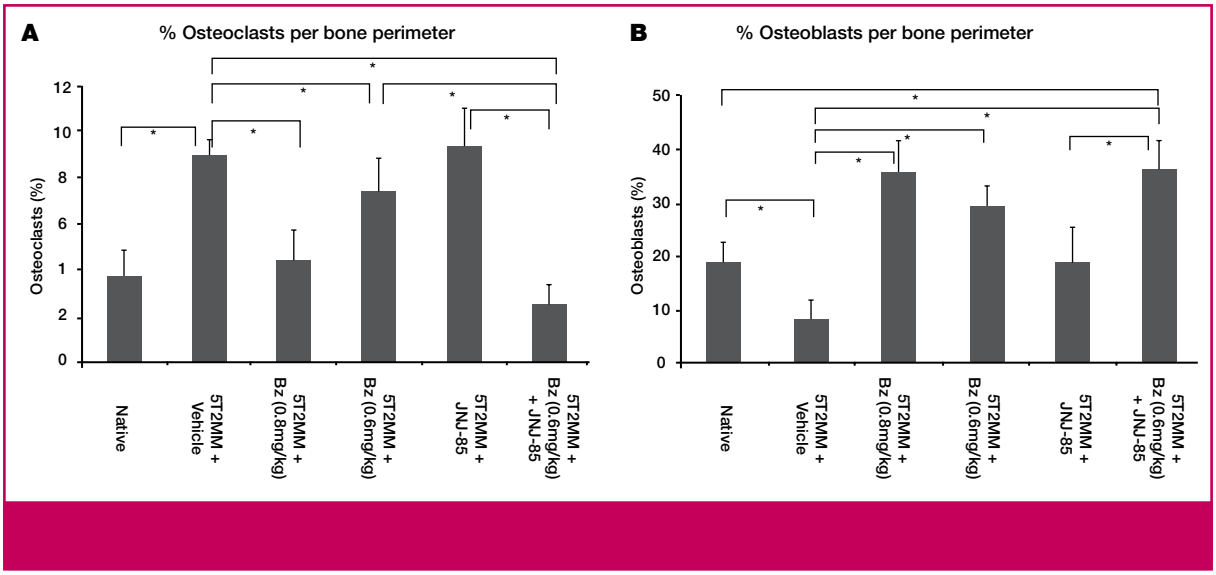

Figure 2. Effect of bortezomib (0.8 mg/kg, twice weekly, s.c.), bortezomib (0.6 mg/kg, twice weekly, s.c.), JNJ-26481585 (1.25 mg/kg, every other day, s.c.) as single agents and the combination bortezomib (0.6mg/kg, twice weekly, s.c.) + JNJ-26481585 (1.25 mg/kg, every other day, s.c.) on development of the multiple myeloma (MM) bone disease in 5T2MM bearing mice. A. % osteoclasts B. % osteoblasts. Mean values, SE and six groups of 10 mice are shown (* $p<0.05$).

number and the percentage osteoclasts in mice treated with JNJ-26481585 were significantly reduced when compared to vehicle-treated mice, and returned to similar level to that seen in naive non-tumour-bearing mice ($p<0.05$).[6]

Part II: Bortezomib in combination with JNJ-26481585: an in vivo study

Therapeutic effect of bortezomib as single agent

In a therapeutic setting using the 5T2MM mice, treatment with bortezomib (0.8 mg/kg, twice weekly, s.c.) resulted in a reduction of BM plasmacytosis of 98% and no paraprotein could be detected with electrophoresis in the treated group ($p<0.0001$). Bortezomib treatment of the tumour-bearing mice also resulted in a near-normalisation of microvessel density in the BM and a decrease in the MM bone disease. This reduction in the microvessel density and MM bone disease could not only be a consequence of the reduction of tumour burden but also of direct effects of bortezomib on endothelial cells, osteoblasts and osteoclasts ($p<0.05$).[7]

Therapeutic of bortezomib in combination with JNJ-26481585

We combined bortezomib (0.6 mg/kg, twice weekly, s.c.) at a concentration where 99% of the tumour load was reduced in the 5T2MM model ($p<0.05$) and bone disease was only reduced partially ($p<0.05$), using a suboptimal dose of JNJ-26481585 (1.25 mg/kg, every other day, s.c.). 5T2MM mice treated with JNJ-26481585 at this low dose showed no significant reduction in the tumour burden in the BM ($p>0.05$) and did not reduce the bone disease significantly. As we observed a reduction of almost 100% in the tumour load when bortezomib was used as single agent, we could not observe any additive reduction on this parameter with the combination of both drugs. Despite this lack of additional effect on tumour burden, we did observe a more pronounced reduction of osteoclasts and increase of osteoblasts, trabecular bone volume and trabecular number in the combination treatment compared to their effect as single agents. The percentage osteoclasts and osteoblasts are shown in *Figure 2*.[7] Since tumour load was completely reduced when using bortezomib alone, we can exclude in the combination therapy that additional effects on tumour load affected the MM bone disease indirectly. This indicates that the enhanced effects in the combination therapy on the bone parameters are mainly caused by direct effects of both compounds on the microenvironment.

Conclusion

These data suggest that JNJ-26481585 has a potent

anti-MM activity by reducing tumour burden, angiogenesis and the MM bone disease. Furthermore similar results could be obtained in a prophylactic and therapeutic setting, indicating that JNJ-26481585 as single agent could overcome the protective signals from the BM microenvironment towards the malignant cells. This demonstrates the importance for further clinical evaluation of JNJ-26481585 in multiple myeloma patients. In the second part of the thesis we demonstrated that the bone remodelling properties of bortezomib could be improved when combined with low dose of the HDAC-inhibitor JNJ-26481585. This suggests that the combination strategy could be a useful strategy for the treatment of MM patients, especially in those patients with skeletal complications.

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