Effects of cabozantinib alone and in combination with bortezomib in the 5TGM1 murine multiple myeloma model

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Introduction

Cabozantinib (cabo) inhibits tyrosine kinases including MET, VEGF receptors, and AXL, and has clinical activity in patients with advanced renal cell cancer and other solid tumors with bone metastases (1-3). Multiple myeloma (MM) is a monoclonal B-cell (plasma cell) neoplasia representing ~2% of all cancer deaths. The clinical hallmark is presence of multiple osteolytic lesions causing bone pain, pathologic fractures, and hypercalcemia. Circulating levels of HGF and VEGF are upregulated in MM patients (4), and regulation of plasma cell-osteoblast communication by the HGF-MET signaling pathway has been implicated in the development of lytic bone disease in these patients (5).

Aim of the Study

We studied the efficacy of cabo in the 5TGM1 murine multiple MM alone and in combination with bortezomib (brtz).

Materials and Methods

Female C57BL/KaLwRij mice, 6-7 weeks old, were allocated to treatment groups (n=15-16 per group) with equivalent average body weights. At day 0, animals were inoculated with 5TGM1 mouse myeloma cells by IV administration. Dosing began at day 1 and continued daily until euthanasia. Two daily doses of cabo, 10 and 30 mg/kg, were tested in a 35-day study, and the 10 mg/kg dose was chosen to a 70-day survival study including a combination treatment group with brtz. Analgesia (buprenorphine 0.02 mg/ml in the drinking water) was used when signs of pain were observed. The mice were sacrificed when weight loss over 20%, paraplegia or breathing problems were observed, or at the end of the study. Serum IgG2b (ELISA kit, Bethyl Laboratories Inc, Montgomery, TX, USA), and TRACP 5b (TRACP 5b; MouseTRAP kit, IDS, Boldon, UK) were measured before and 2, 3 and 5 weeks after the inoculation and in the survival study also at sacrifice. Development of osteolytic lesions was detected by X-ray (Faxitron MX-20 D12, Faxitron Corp. Illinois, USA) at day 35 and in the survival study also at sacrifice. In the survival study, mice were sacrificed individually when they became paraplegic, lost over 20% of body weight, or had severe breathing problems. Statistical analysis: biochemical markers up to day 35 were analyzed with LME model with day -1 values as baseline; osteolytic area with ANOVA followed by Tukey's HSD test; number of osteoclasts and necrotic tumor area with Kruskal-Wallis followed by Mann-Whitney U-test; survival with log-rank test of Kaplan-Meier estimates.

Bone analysis

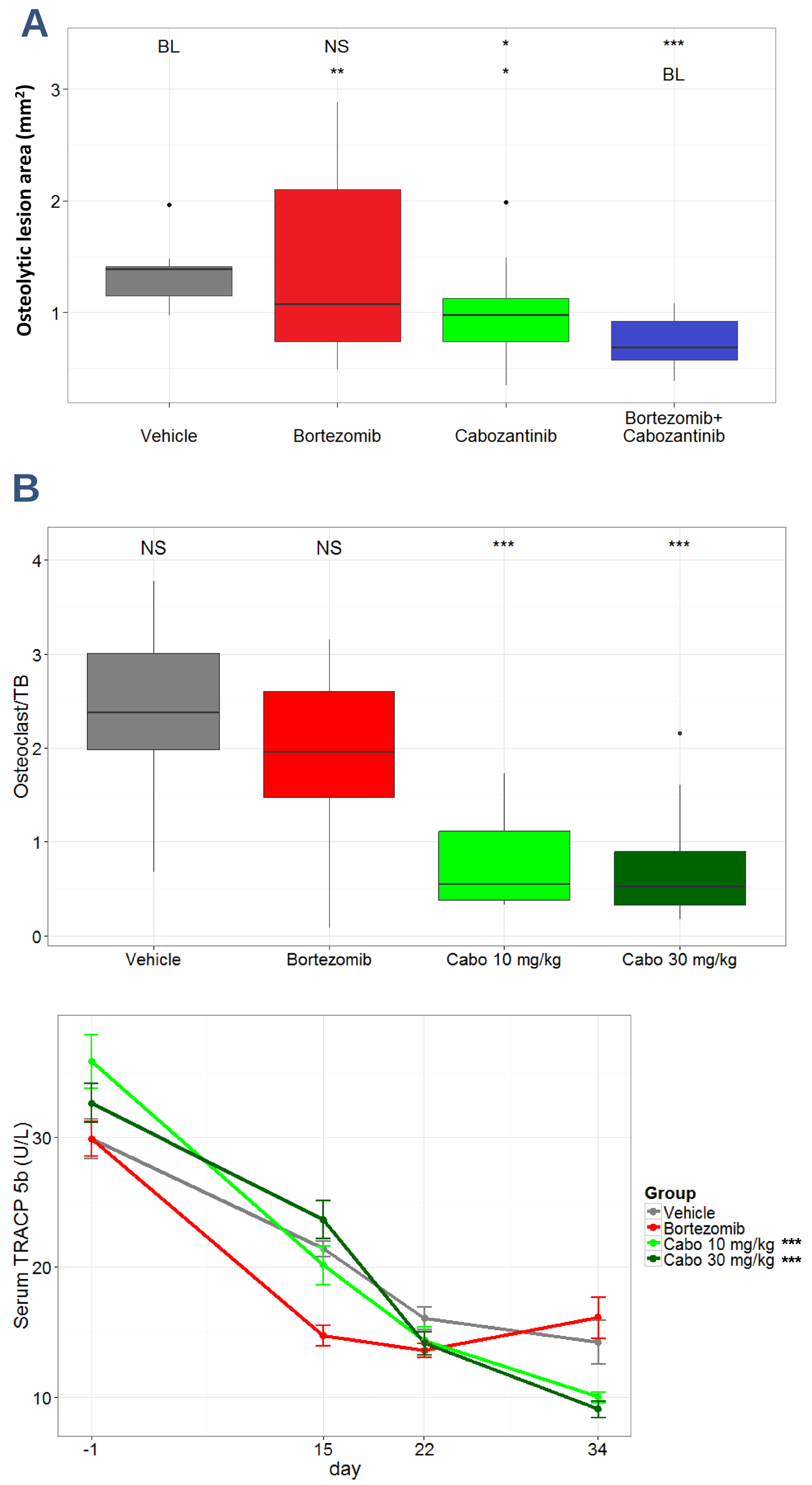
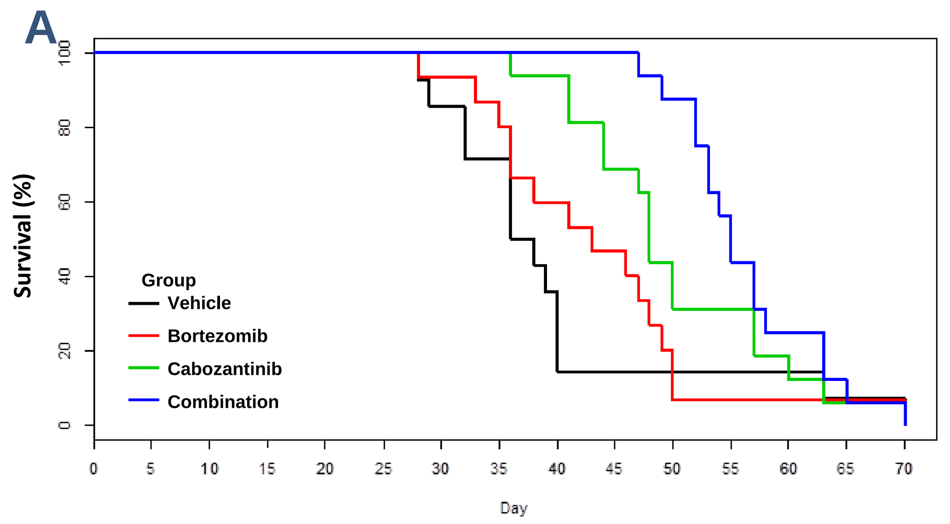


FIGURE 1. A) Total osteolytic area at day 35 was determined from X-ray images (mm², median±IQR25%±min/max). Outliers are marked as floating points in the figure but they were not removed in the statistical analysis. Cabo monotherapy decreased total osteolytic area, and the effect was more pronounced with combination therapy. **B)** Number of osteoclasts relative to the tumor-bone interface length 35 days after the inoculation (#/mm, median±IQR25%±min/max). **C)** Bone resorption was measured as secreted TRACP 5b into serum during the 35-day study (U/L, mean±SEM). Cabozantinib decreased serum TRACP 5b levels compared to control group.

Survival analysis



B

	Vehicle	Bortezomib	Cabozantinib	Combination
Median survival (days)	37	43	48	55
adj. p-value vs vehicle	BL	0.340	0.090	0.049
adj. p-value vs combination		0.005	0.280	BL
Hazard ratio vs vehicle	BL	0.70	0.47	0.42
95% CI vs vehicle	BL	0.331-1.475	0.221-0.997	0.197-0.882
Hazard ratio vs combination		3.16	1.64	BL
95% CI vs combination		1.477-6.762	0.815-3.303	BL

FIGURE 2 A) Kaplan-Meier curve of survival. **B)** Median survival and hazard ratios compared to vehicle and combination therapy. Cabo + brtz combination therapy significantly increased overall survival. BL= baseline (comparator group).

Tumor analysis

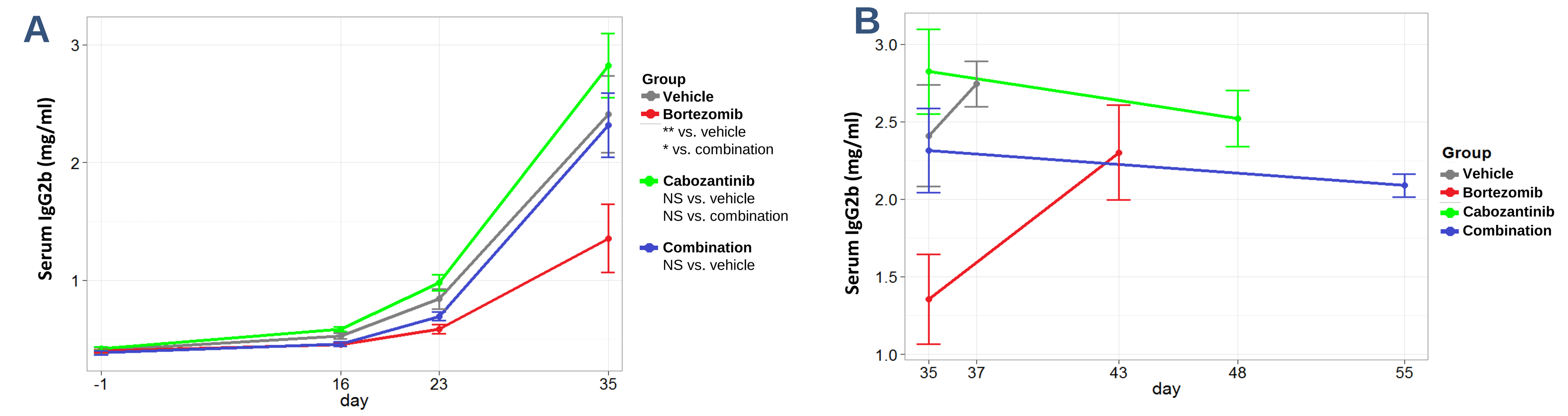


FIGURE 3. A) Secreted IgG2b in serum was measured as a tumor marker (mg/ml, mean±SEM). Brtz decreased serum IgG2b levels compared to vehicle group. **B)** Change of serum IgG2b from day 35 to sacrifice in the survival study. Median survival of the group is used as the sacrifice timepoint. IgG2b increased in vehicle and brtz groups, but decreased in cabo and combination groups

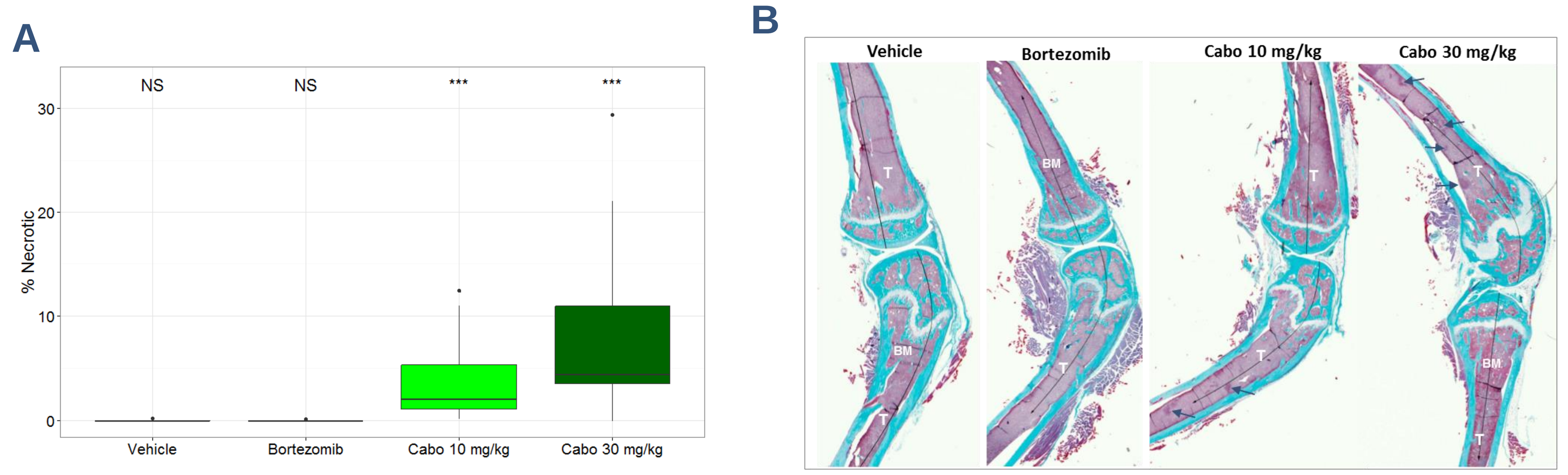


FIGURE 4. A) Necrotic tumor area relative to total tumor area (%, median±IQR25%±min/max) were determined histomorphometrically. Cabozantinib decreased osteoclast number and increased necrotic tumor area. **B)** Representative images of Masson-Golder Trichrome –stained sections. T= tumor, BM= bone marrow, arrows = necrosis. *** = p < 0.001, NS = Non-significant.

Summary

- In both studies, by study day 35 the osteolytic lesions were not affected by brtz, were reduced by cabo alone, and further reduced by the combination treatment.
- Also by day 35, brtz had inhibited the rise in serum IgG2b levels, but cabo and the combination treatment had not.
- Despite the effects on serum IgG2b, brtz did not significantly increase survival, whereas cabo and the combination treatment did. Increased survival with the combination was significant when compared to brtz monotherapy, but not when compared to cabo monotherapy.
- Histology of the 35-day study showed that cabo dose dependently increased the necrotic tumor area in bone. We hypothesized that the rise in IgG2b was due to lysis of plasma cells and not tumor growth. Consistent with this hypothesis, the IgG2b levels of cabo treated mice decreased from day 35 onwards in the survival study.

Conclusions

In summary, cabo increased survival and exhibited bone-protective and anti-tumor effects in this murine model of MM. Combination with brtz showed additive effects on survival. Based on these results, further investigation of cabo in combination with other therapies in multiple myeloma is warranted.

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