Effects of Cabozantinib in the 5TGM1 murine multiple myeloma model

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Introduction

Cabozantinib, an inhibitor of tyrosine kinases including MET, VEGFR2, and RET, has shown activity in preclinical bone metastasis tumor models (1-3), and clinical activity in patients with castration-resistant prostate cancer and bone metastases (4). Multiple myeloma (MM) is the second most common hematologic malignancy, and represents ~2% of all cancer deaths. MM is a monoclonal B-cell (plasma cell) neoplasia with clinical hallmarks of multiple osteolytic lesions causing bone pain, pathologic fractures, and hypercalcemia. Upregulated HGF and MET are associated with aggressive disease in MM (5), 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 (6). Despite availability of active therapies such as bortezomib, lenalidomide, and carfilzomib, MM is generally thought to be incurable, and therefore new treatment options are needed.

Aim of the Study

Our aim was to determine the activity of cabozantinib on bone lesions and tumor burden in the syngeneic 5TGM1 mouse MM model (7).

Materials and Methods

Four experimental groups were included: negative control group receiving vehicle, positive control group receiving bortezomib (Bz, 0.5 mg/kg ip twice a week), low dose cabozantinib group (Cabo 10 mg/kg, PO QD) and high dose cabozantinib group (Cabo 30 mg/kg, PO QD). 7 weeks old Female C57BL/KaLwRij mice were allocated to treatment groups (n=15 per group) with equivalent average body weights. On day 0, animals were inoculated with 5TGM1 mouse myeloma cells by IV administration. Dosing began on day 1 and continued daily until euthanasia at day 35. Body weights were determined twice a week and blood samples were collected on days -1, 15, 22, and 34 for analysis of paraprotein (IgG2b) and tartrate-resistant acid phosphatase isoform 5b (TRACP 5b). The development of osteolytic lesions was detected by radiography at the end of the study. The mice were sacrificed 5 weeks after inoculation, examined macroscopically, and their bones were collected for histomorphometric analysis. 4/15 mice were euthanized before the end of the experiment due to paraplegia in control and bortezomib groups, but none in cabozantinib groups. Animals euthanized within four days of the end of the experiment were included in the analysis.

Frequency of soft tissue lesions

Organ		Control	Bz	Cabo 10 mg/kg	Cabo 30 mg/kg
Spleen	%	15.4	0.0	20.0	7.0
	p-value		0.222	1.000	0.583
Kidneys	%	3.8	0.0	3.3	0.0
	p-value		0.481	1.000	0.464
Ovaries	%	57.7	7.1	3.3	0.0
	p-value		<0.001	<0.001	<0.001
Other	count	9.0	1.0	3.0	0.0
	p-value		0.001	0.020	<0.001

Table 1. Frequency of visceral tumors based on macroscopic autopsy findings expressed as percentage of organs with a tumor out of the total number of organs examined. Bortezomib and cabozantinib decreased the frequency of soft tissue tumors

Biochemical markers

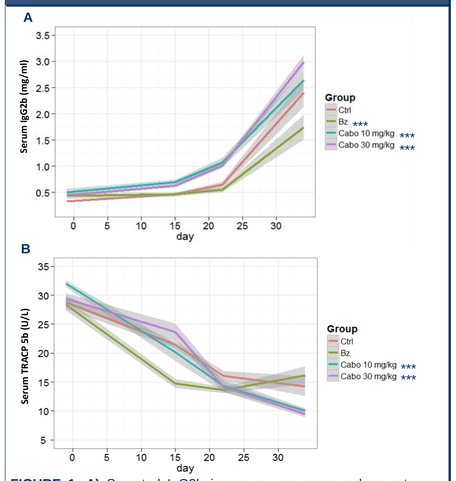

FIGURE 1. A) Secreted IgG2b in serum was measured as a tumor marker during the study (mg/ml, mean±SEM). Bortezomib decreased and Cabozantinib increased serum IgG2b levels compared to control group. **B)** Bone resorption was measured as secreted TRACP 5b into serum during the study (U/L, mean±SEM). Cabozantinib decreased serum TRACP 5b levels compared to control group. Statistical analysis of IgG2b and TRAP 5b were performed using LME model where day -1 values were included as baseline. *** = p < 0.001.

Histomorphometric analysis

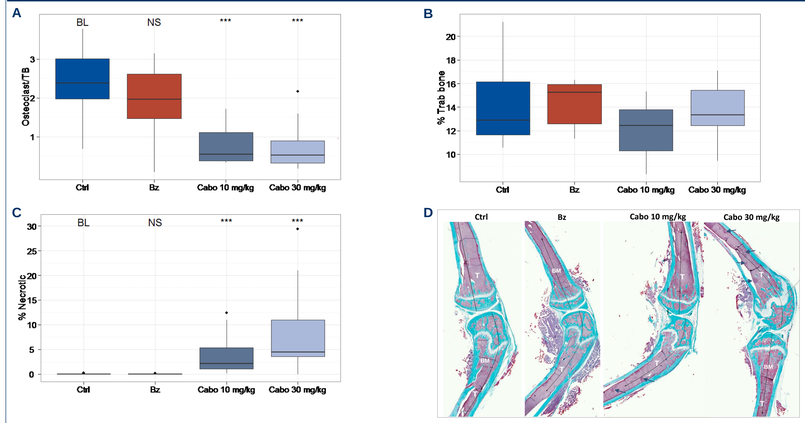

FIGURE 2. A) Number of osteoclasts relative to the tumor-bone interface length (#/mm, median±IQR25%±min/max), **B)** total bone area relative to ROI area (%, median±IQR25%±min/max) and **C)** necrotic tumor area relative to total tumor area (%, median±IQR25%±min/max) were determined histomorphometrically. Cabozantinib decreased osteoclast number and increased necrotic tumor area. **D)** Representative images of Masson-Golder Trichrome –stained sections. T= tumor, BM= bone marrow, arrows = necrosis. *** = p < 0.001, NS = Non-significant.

Radiographic analysis

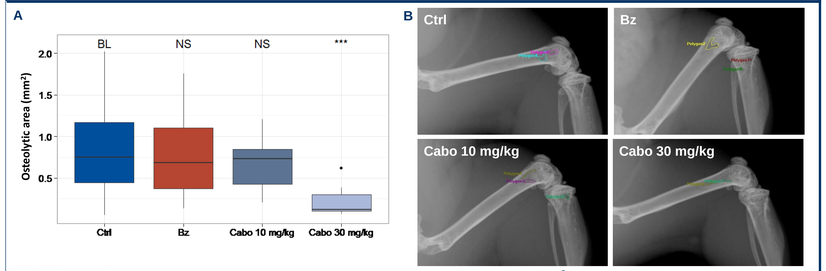

FIGURE 3. A) Total osteolytic area at sacrifice was determined from X-ray radiography (mm^2, median±IQR25%±min/max). Outliers are marked as floating points in the figure but they were not removed in the statistical analysis. Cabozantinib dose 30 mg/kg decreased total osteolytic area. *** = p < 0.001. Statistical analysis was performed using ANOVA and Tukey's HSD test. **B)** Representative X-ray images of each treatment group visualizing the analysis of osteolytic lesions. Each polygon represents one lesion. The sum of areas represent total osteolytic area in each animal.

Summary

- Cabozantinib exhibited bone protective effects: mean and total area of osteolytic lesions were reduced at the 30 mg/kg dose, and serum TRACP 5b values and osteoclast counts at the tumor-bone interface were reduced at both doses. However, relative bone area did not differ from control according to histomorphometry.
- Cabozantinib induced earlier increase in IgG2b levels, but IgG2b at sacrifice did not differ from control. Cabozantinib dose-dependently increased the necrotic tumor area in bone, indicating the possibility that the rise in IgG2b may have been due to lysis of plasma cells.
- Cabozantinib decreased the frequency of soft tissue lesions.
- Cabozantinib appeared to prevent premature euthanasia due to paraplegia or other morbidity. Although the study was not designed as a survival study, the difference in time to sacrifice was statistically significant.
- Bortezomib reduced serum IgG2b levels and decreased the frequency of soft tissue lesions, but did not show bone protective properties.

Conclusions

In summary, cabozantinib showed both bone-protective and anti-tumor effects in this murine model of multiple myeloma. Based on these results, further investigation of cabozantinib in multiple myeloma is warranted.

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References

1. Nguyen, et al. (2013) PLoS One, 8(10): e78881.
2. Dai, et al. (2014) Clin Cancer Res, 20(3): 617-630.
3. Graham, et al. (2014) J Natl Caner Inst, doi:10.1093/jnci/dju033 [Epub ahead of print March 14, 2014]
4. Smith, et al. (2013) J Clin Oncol, 31(4): 412-419.
5. Rocci, et al. (2014) Br J Haematol, 164(6): 841-850.
6. Standal, et al. (2007) Blood, 109(7): 3024-3030.
7. Garret et al. (1997) Bone, 20(6): 515-520.