Pharmacokinetic/Pharmacodynamic Characterization of Three Efficacious Cathepsin K Inhibitors on the Bone Resorption Marker CTX-I in Cynomolgus Monkeys

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Abstract

Cathepsin K (CK) inhibitors are in clinical development for the treatment of osteoporosis. During Medivir's cathepsin K inhibitor program, we have evaluated the pharmacokinetic/pharmacodynamic (PK/PD) relationship of three novel, potent and selective cathepsin K inhibitors, namely MV074840, MV074942 and MV076159, in cynomolgus monkey *in vivo*. Human monocyte-derived osteoclasts were used to assess anti-resorptive potency *in vitro*. Conscious, male cynomolgus monkeys aged 2-3 years were dosed with inhibitor or vehicle orally (3 – 30 µmol/kg) or intravenously (4.6 µmol/kg). Plasma samples were collected at regular intervals for assessment of compound exposure levels. Efficacy was assessed by monitoring plasma levels of CTX-I, a selective biomarker of collagenous bone resorption, using CTX-I ELISA. An indirect response model correcting for fluctuations in diurnal CTX-I levels was used to assess the PK/PD relationship for the inhibitors. The three inhibitors characterized, MV074840, MV074942 and MV076159, had K_i values of 1.6, 1.5 and 0.9 nM respectively on human cathepsin K enzyme assays and IC_{50} values of 35, 44 and 34 nM respectively on osteoclasts.

Oral administration of the compounds to cynomolgus monkeys produced dose-dependent reductions of CTX-I. MV076159 was most efficacious with a 95% drop in CTX-I levels 8 hours after a dose of 30 µmol/kg (baseline CTX-I: 1.90±0.39 ng/mL vs MV076159: 0.097± 0.08 ng/mL, n = 4, p<0.05). The inhibitory effect of MV076159 was still prominent 24 h after a single dose (75% inhibition) despite the lack of plasma exposure at this time point. CTX-I levels returned to baseline after a single dose, indicating full reversibility. PK/PD analysis of the three compounds revealed excellent *in vivo* potency with plasma IC_{50} values ranging between 10-20 nM.

The cathepsin K inhibitors evaluated are highly efficacious in cynomolgus monkey *in vivo*, displaying outstanding potency and sustained, and reversible, efficacy based on biomarkers. This *in vivo* profile is possibly linked to long-lasting osteoclast inhibition due to the lysosomotropic properties of the compounds. Due to these promising properties, the compounds are attractive for progression into clinical development.

Introduction

Osteoporosis results from excessive bone degradation. It is characterized by low bone mass and deterioration of skeletal tissue architecture which can lead to bone fragility and predisposes an individual to an increased risk of fracture.

Cathepsin K is a lysosomal cysteine protease expressed abundantly in osteoclast cells. Numerous lines of evidence support a pivotal role for cathepsin K in bone degradation. Indeed, cathepsin K inhibitors decrease markers of bone resorption and increase bone mineral density (BMD) in man. These anti-resorptive effects appear to occur without negatively impacting bone formation, differentiating this potential osteoporosis treatment from currently available anti-resorptives such as bisphosphonates. This highlights the potential for cathepsin K inhibition as a novel therapeutic approach for bone metabolism diseases such as osteoporosis.

Medivir has developed three potent, and highly selective, cathepsin K inhibitors. The aim of the current study was to evaluate their efficacy and pharmacokinetics in conscious cynomolgus monkeys *in vivo*.

Methods

- The potency and selectivity of the inhibitors were determined using recombinant human cathepsins K, S, L, H, F, V and B
- Functional reversibility of the inhibitors against cathepsin K was assessed
- Cellular inhibition of cathepsin K was monitored using a human osteoclast system as previously described (Fuller *et al.*, 2006)
- Iip10 Accumulation, which reflects inhibition of cellular cathepsin S activity, was measured in a human EBV-B-cell line
- Conscious cynomolgus monkeys were dosed p.o. or i.v. with cathepsin K inhibitor or corresponding vehicle between 7.00 a.m. and 9.00 a.m. Blood samples were drawn at various time points after dosing. Plasma samples were collected for analysis of compound levels and CTX-I.
- The C-terminal degradation product of collagen type I (CTX-I) in plasma was measured using a commercially available kit (CrossLaps, IDS Nordic A/S, Herlev, Denmark)
- Compound levels in plasma were determined using reverse–phase liquid chromatography and electrospray tandem mass spectrometry (LC-MS-MS)

Potency and Selectivity *in vitro*

Human enzyme level:

Assay	MV074840	MV074942	MV076159
Cathepsin K	1.7	1.6	0.75
Cathepsin S	5800	14000	19000
Cathepsin L	510	1600	1800
Cathepsin B	510	1200	1300
Cathepsin H	>10000	>10000	>10000
Cathepsin V	2700	1700	4000
Cathepsin F	470	1700	2800

All values are given as K_i values in nM

- MV074942 and MV076159 display more than 1000-fold selectivity vs related cysteine proteases
- The three inhibitors bind reversibly to cathepsin K enzyme (e.g. K_{off} provides a half-life of 90 s for MV076159)

Cellular level:

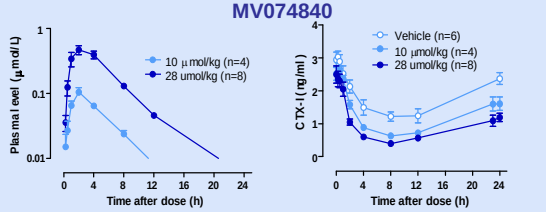
Assay	MV074840	MV074942	MV076159
Human osteoclasts	35	44	34
Iip10 accumulation	Not determined	48000	23000

All values are given as IC_{50} values in nM

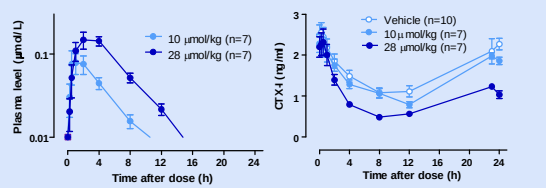
- MV074942 and MV076159 display approximately 1000-fold selectivity at the cellular level

PK and Efficacy *in vivo*

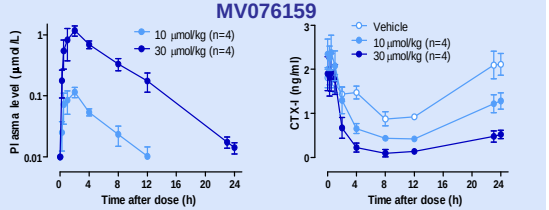
MV074840



MV074942



MV076159

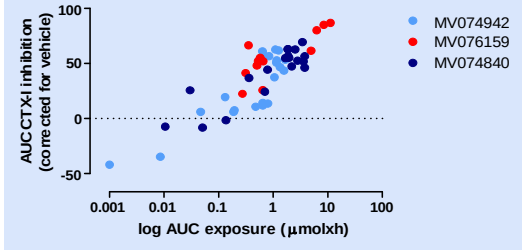


In vivo efficacy summary:

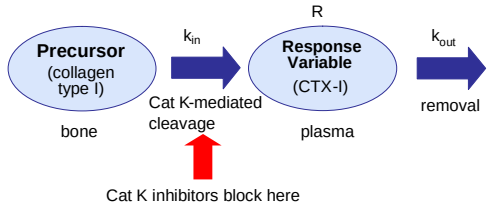
Treatment	Dose (µmol/kg)	Max inhibition (%)	Inhibition at 24h (%)	Inhibition at 48h (%)
Vehicle		50	0	0
MV074840	28	84	52	-5
MV074942	28	75	51	3
MV076159	30	95	75	22

- Significant reductions of CTX-I are present 24h after dose of cathepsin K inhibitor, despite minimal plasma exposure at this time point
- Effects of inhibitors are fully reversible

Exposure vs Effect Relationship



Degree of efficacy over 24h is related to compound exposure over 24h



An indirect response model was used to characterize compound potency *in vivo*. Diurnal changes in CTX-I levels were corrected for. Equation:

$$\frac{dR}{dt} = kin \cdot \left(\frac{1 - Cp^n}{IC_{50} \cdot Cp^n}\right) - kout \cdot R$$

$$kin = Rm \cdot (1 + Ramp \cdot \cos(2 \cdot \pi \cdot (T - Tz)/24))$$

Plasma IC_{50}s for all three compounds ranged between 1 – 10 nM. This higher degree of potency *in vivo* compared to osteoclast data *in vitro* is probably due to a prolonged action at osteoclasts *in vivo* – the desired site of action.

Summary and Conclusions

- The three lysosomotropic inhibitors described are potent and highly selective inhibitors of human cat K *in vitro*
- Advantageous lysosomotropic properties of these compounds lead to no loss of selectivity at the cellular level coupled with enhanced potency in an osteoclast cell-based assay (Fuller *et al.*, 2009)
- The compounds are well-tolerated and inhibit circulating CTX-I levels by up to 95% in cynomolgus monkey *in vivo*
- Efficacy duration exceeds plasma exposure, likely due to a prolonged residence time in osteoclasts – the intended site of action
- The high potency and prolonged efficacy duration *in vivo* together with excellent selectivity renders these compounds attractive candidates for clinical development

References

Kirstein B, Grabowska U, Samuelsson B, Shiroo M, Chambers TJ, Fuller K. A novel assay for analysis of the function of human osteoclasts. *J Transl Med* 2006; 4: 4553.

Fuller K. Lindstrom E, Edlund M, Henderson I, Grabowska U, Samuelsson B, Chambers TJ. The resorptive apparatus of osteoclasts supports lysosomotropism and increases potency of lysosomotropic versus non-lysosomotropic inhibitors of cathepsin K. ASBMR 2009, MO0225.