

A first-in-human Phase I study of 4SC-201, an oral histone deacetylase (HDAC) inhibitor, in patients with solid tumors

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Background

Histone deacetylase (HDAC) enzymes are considered to be among the most promising targets in drug development for cancer therapy. Various HDAC inhibitors are currently under clinical investigation in a broad range of tumor entities including both hematological malignancies and solid tumors.

4SC-201 (former code BYK408740) is a newly developed, specific, potent, pan-HDAC inhibitor with a favorable preclinical profile as demonstrated by an intensive *in-vitro* and *in-vivo* pharmacological, pharmacodynamic and toxicological test program.

Objectives

Primary objectives:

- To investigate safety and tolerability of repeated ascending oral doses of 4SC-201 and to determine the potential maximum tolerated dose (MTD) and dose-limiting toxicities (DLT)
- To assess the pharmacokinetics of 4SC-201

Secondary objectives:

- To evaluate optimal dose scheduling for further studies
- To assess pharmacodynamic effects (HDAC enzyme inhibition, histone acetylation) of 4SC-201
- To evaluate potential anti-cancer activities of the drug

Study Design and Methods

Study design:

- Open label, inter-patient dose-escalating, single-centre study
- Four treatment cycles each consisting of repeated oral doses for 5 consecutive days followed by a 9-day rest period
- Five sequential dose cohorts with a starting dose of 100 mg once-daily (QD); 50 or 100% dose increments up to the highest dose level of 800 mg
- Three to six patients to be included at each dose level depending on the evaluation of safety and tolerability

Main eligibility criteria:

Inclusion:

- Male and/or female patients, age ≥ 18 years
- Histologically or cytologically documented diagnosis of primary or metastatic solid tumours refractory to standard therapy or for which no standard therapy exists
- At least one evaluable lesion (by CT-scan, MRI, or bone scan)
- Progressive disease as defined by new or progressive lesions on CT-scan, MRI, bone scan or by rising PSA
- ECOG Performance Status 0-1

Exclusion:

- Prior treatment with other HDAC inhibitors
- Anti-cancer therapy including chemotherapy, radiotherapy, endocrine therapy, immunotherapy or use of other investigational agents within the last 4 weeks or a longer period depending on the defined characteristics of the agents used
- Serious concomitant systemic disorders (e.g. active infection) that, in the opinion of the investigator, would compromise patient safety or compromise his/her ability to complete the study
- Presence of metastatic disease that, in the opinion of the investigator, would require palliative treatment within 4 weeks of enrolment
- History or current evidence of clinically relevant allergies or idiosyncrasy to drugs or food
- Symptomatic brain metastases
- Significant cardiovascular disease; current evidence of any severe internal, psychiatric or neurologic disease
- History of allergic reactions attributed to compounds of similar chemical or biological composition to the study drug
- Major surgery within the last 4 weeks

DLT:

- Toxicity is graded during cycle 1 using CTCAE Version 3.0
- Non-hematological grade 3 or 4 toxicity with the exceptions of alopecia, diarrhea, rash, arthralgias and myalgias, nausea and vomiting (despite maximum treatment)
- Grade 4 neutropenia lasting > 5 days or associated with fever > 38.5 °C
- Grade 4 thrombocytopenia

Study Results

First oral administration of 4SC-201 to humans started in September 2007. The last patient completed the main phase of the study after four treatment cycles in January 2009.

Demographic Data

- 18 pts (9M/9F), median age 58 yrs (range 40–70) were treated at five dose levels: 3 pts each at 100 mg, 200 mg, 400 mg, 600 mg and 6 pts at 800 mg. All 18 pts received at least 2 treatment cycles and were evaluable for safety and toxicity assessment.

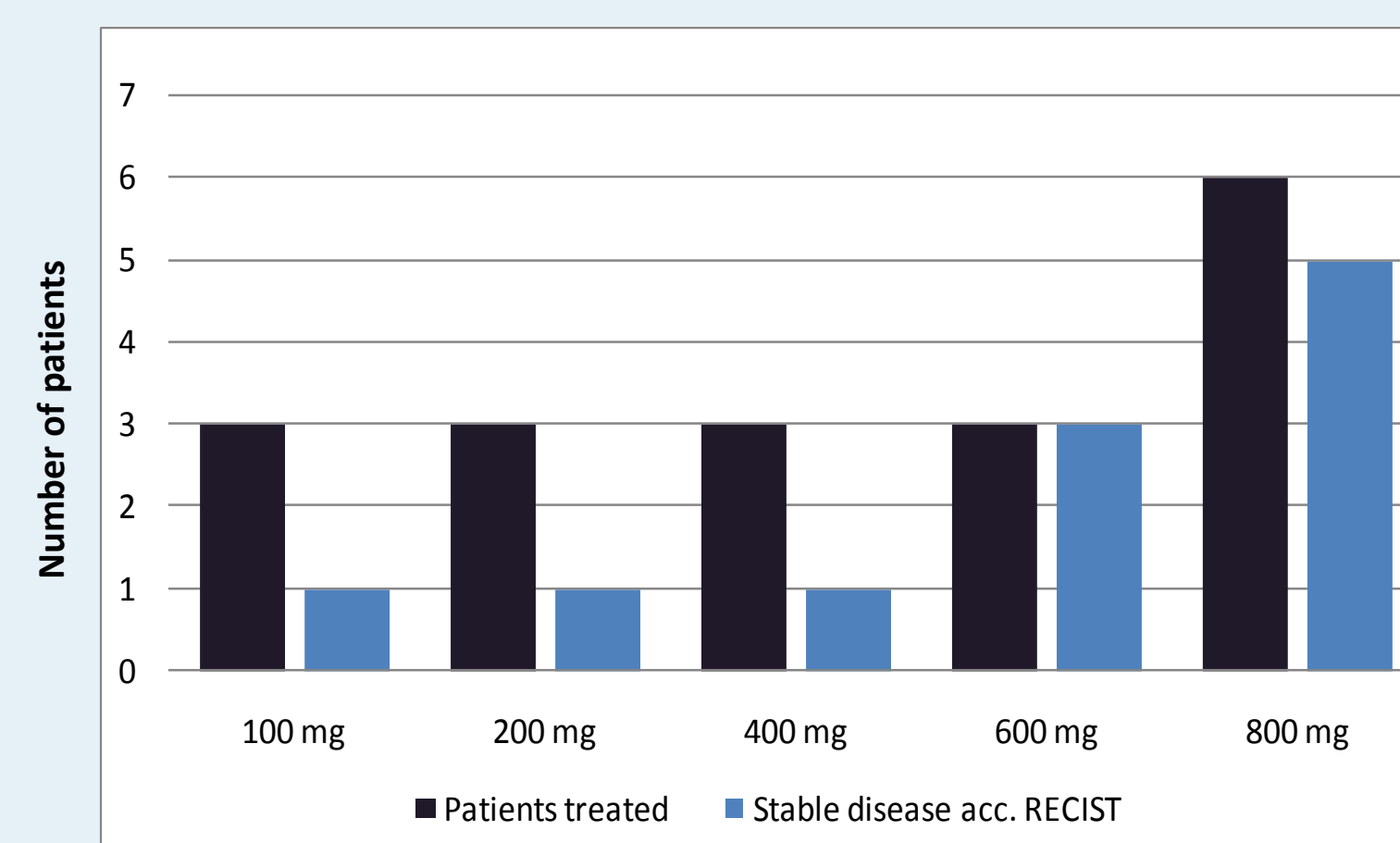
Table 1: Baseline patient and disease characteristics

Characteristics	N = 18
Mean age (range)	56 years (40 – 70)
Gender, N (%)	
Male	9 (50%)
Female	9 (50%)
Tumor indication:	
NCLC	3
Ovarian	3
Mesothelioma	2
Colorectal	2
Cholangio carcinoma	1
Sigmoid colon carcinoma	1
Retropertoneal liposarcoma	1
Thymoma	1
Pancreatic	1
Endometrium carcinoma	1
Malignant melanoma	1
Merkel cell carcinoma	1

Tumor Efficacy

- All of these 18 patients had evaluable disease at baseline and at least one post-baseline tumor staging
- Stable Disease acc. to RECIST was observed in 11/18 patients after 3 or 4 treatment cycles, thereof 8/9 patients at the two highest dose levels.
- Three patients with SD entered the follow-up treatment phase. One patient with metastatic ovarian cancer and one patient with metastatic thymoma (minor response) continued treatment until cycle 7 and 19, respectively, and stopped both without evidence of progressive disease. The third patient with advanced liposarcoma remains stable on treatment now at 400 mg daily dose for over a year.

Figure 1: Patients with Stable Disease per dose cohort



Safety & Tolerability

- 4SC-201 was generally well-tolerated
- None of the patients discontinued or had to be withdrawn due to drug related AEs, except for one patient with dyspnea related to the underlying tumor disease.
- The AE profile appeared to be dose-dependent, in particular nausea, vomiting and fatigue occurred more often with increasing doses. The intensity of the majority of AEs was mild to moderate.
- Although MTD level was not reached, in some patients dose reduction was necessary after a few cycles of treatment due to cumulative fatigue symptoms.
- Seven patients experienced SAEs, among those two SUSARS occurred (one Grade 3 ALT rise, and hypokalemia); both AEs were judged as likely drug-related.
- Non-suspected SAEs were consistent with those expected for the concerned study population and were mostly related to the underlying tumor disease.
- Overall, no dose-limiting toxicities were seen with regard to cardiac safety in all dose cohorts. A moderate increase of the heart rate during the first hours after drug intake was detected in patients included in the higher dose cohorts. A centralized ECG analysis did not reveal indications of QTc interval prolongation.

Pharmacodynamics

Determination of HDAC Enzyme Inhibition

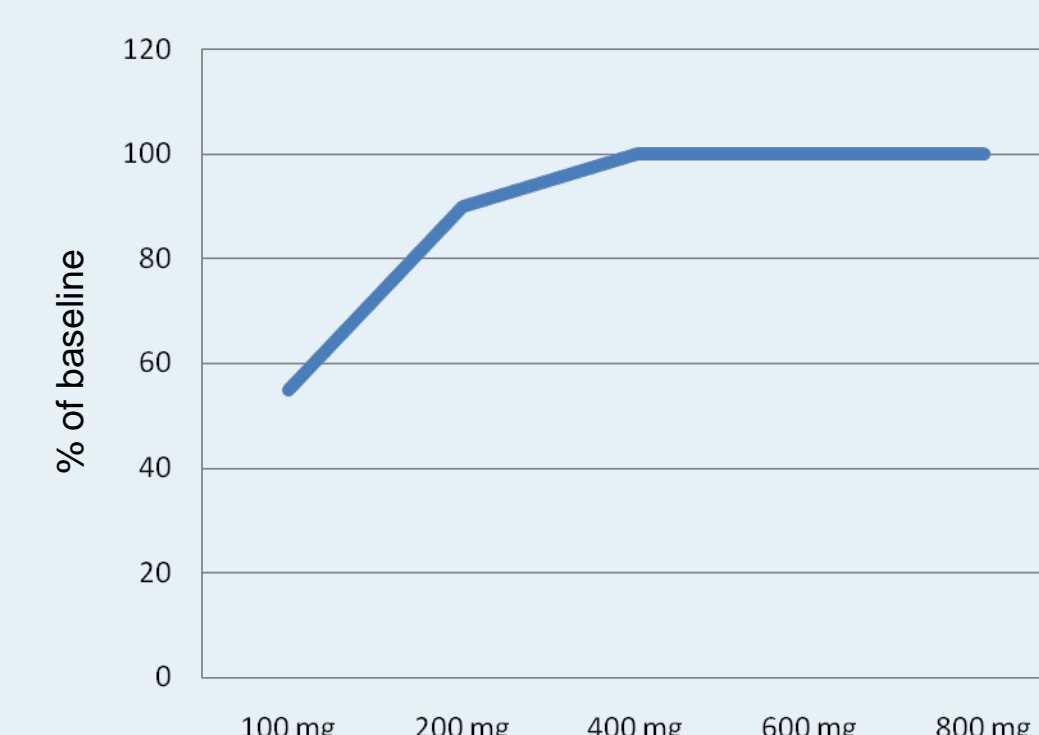
- Objectives:** To determine HDAC enzyme inhibition following administration of 4SC-201

- Rationale:** Dynamic histone modification plays a role in chromatin remodeling. Histone deacetylases (HDACs) are responsible for the reversible histone acetylation. The status of histone acetylation influences the regulation of gene expression.

- Method and Assay Principle:**

The HDAC substrate is added to human whole blood and enters the cells. Deacetylation by HDAC enzymes yields the deacetylated form of the substrate. Lysis of the cells allows contact between the non-cell permeable protease trypsin and the substrate. The deacetylated form of the HDAC substrate serves as a substrate for trypsin resulting in its cleavage. This leads to the release of the fluorogenic dye AMC (Extinction 355 nm, Emission 460 nm).

Figure 2: HDAC Enzyme Inhibition in PBMC vs. dose groups (max, in % of baseline)



- Results:**

As shown in Figure 2, the degree of HDAC enzyme inhibition measured in peripheral blood cells was dose-dependent and ranged from 50% to 100%. The enzyme inhibition was time-dependent and reversible in all dose groups.

Determination of Histone H4 Acetylation Status

- Objectives:** To determine Histone deacetylation status

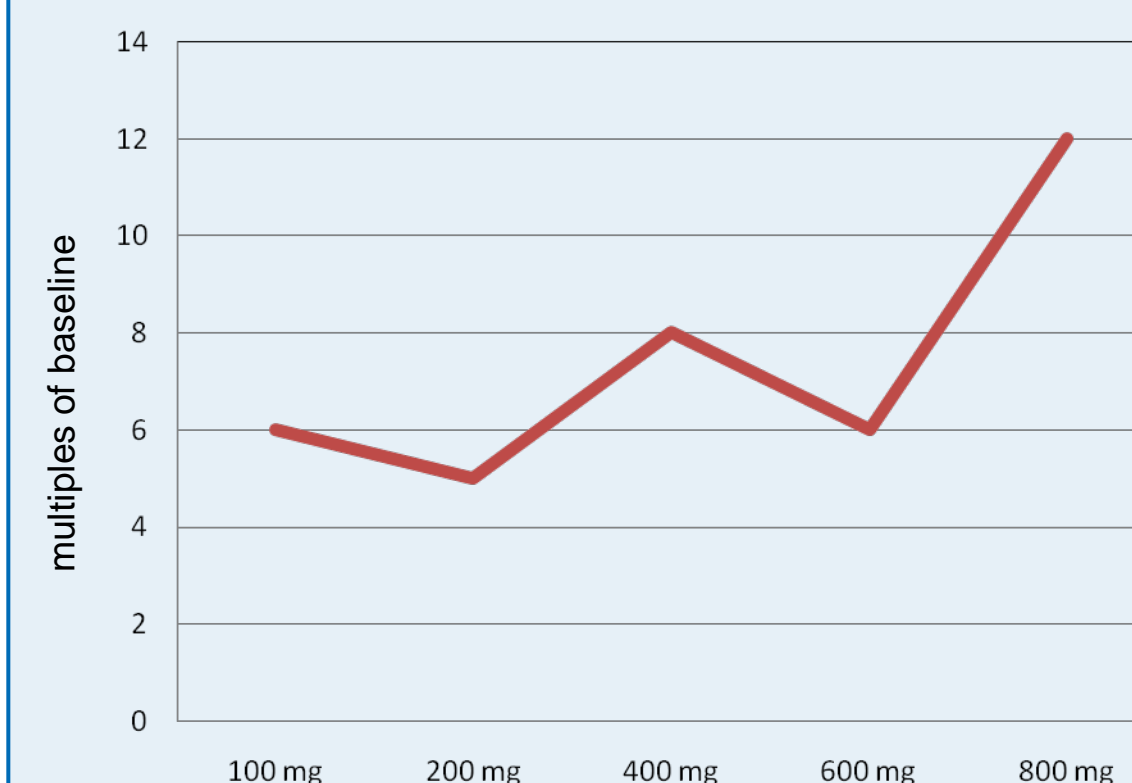
- Rationale:** Chromatin condensation and transcriptional activity is regulated by acetylation of N-terminal lysine residues in core histone proteins H3 and H4.

- Method and Assay Principle:**

The status of histone H4 acetylation on N-terminal residues after treatment of patients during the FIM study was analyzed by Western blotting and a specific antibody hybridisation protocol.

For Western blot analysis PBMCs were lysated with RIPA buffer. Samples were separated on a SDS-PAGE. The proteins were transferred to a PVDF membrane and acetylated H4 histones were detected with specific antibodies. A reprobing of the membranes was performed with a β -actin antibody as a reference for equal protein loading. Finally signal analysis was performed by densitometric scanning of resulting blot signals.

Figure 3: Histone H4 Acetylation Status in PBMC vs. dose groups (max. multiples of baseline value)



- Results:**

As shown in Figure 3, Histone H4 acetylation level increased after dosing, however, this did not differ significantly at dose levels from 100 mg to 600 mg. A remarkable increase was observed at the 800 mg dose level.

References

- Published by Wegener: Development of an enzyme assay for histone deacetylases suited for HTS for the development of HDAC inhibitors (Dissertation, 2003)
- Kelly et al., J Clin Oncol, 23:3923-3931, 2005

Pharmacokinetics

Methods

Plasma samples for PK were analyzed with stand-by bio-analytical investigations on Day 1 (single dose), Day 5 (steady state, after 5 consecutive treatment days in Cycle 1) and Day 47 (steady state, corresponding to the last treatment day in Cycle 4). Pharmacokinetic parameters were determined using traditional non-compartmental analysis. In the following table, steady-state conditions are presented graphically.

Results

Based on the evaluated PK data, dose linearity was observed for all dose levels after single and after repeated dosing. Overall, low inter-individual variability of PK characteristics of 4SC-201 was seen.

Table 2: 4SC-201 mean pharmacokinetic parameters, all dose levels, multiple dose, Day 5

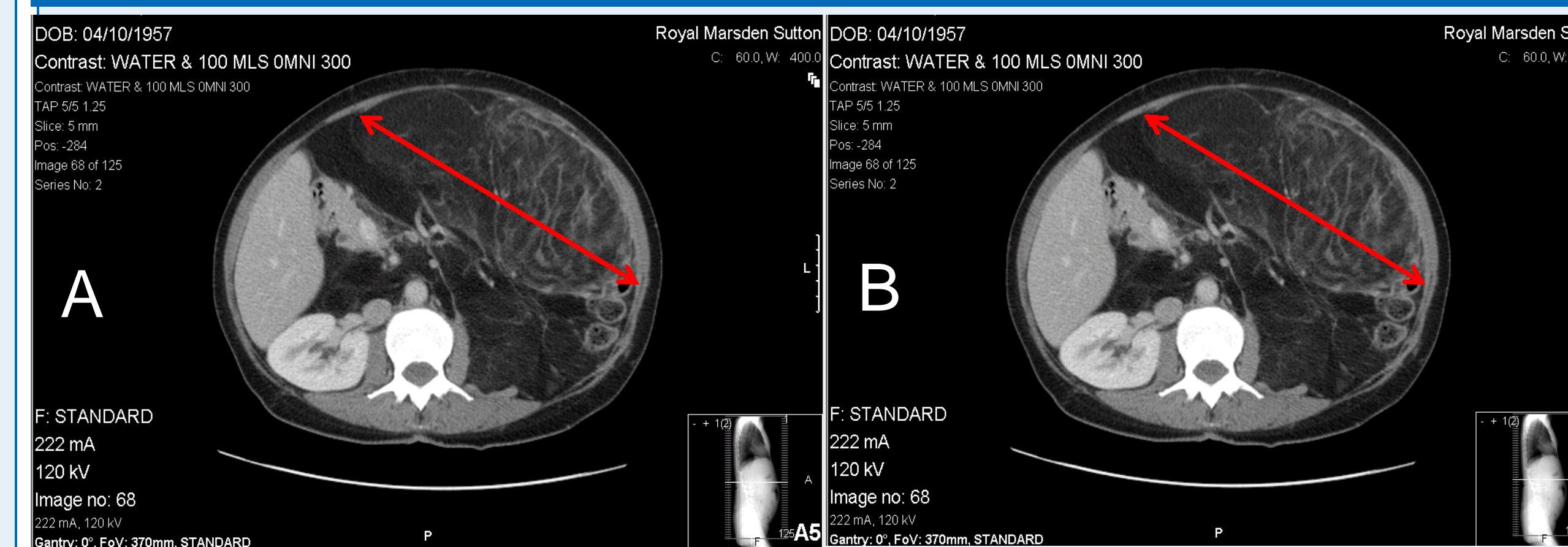
Geometric Mean (Lower 68% Bound, Upper 68% Bound)	4SC-201				
	100 mg	200 mg	400 mg	600 mg	800 mg
N	3	3	3	3	6
t_{max}^a (hr)	2.00 (0.75 - 2.50)	2.00 (1.50 - 5.00)	2.50 (1.50 - 4.00)	2.50 (1.50 - 4.00)	1.75 (1.50 - 6.00)
C_{max} (ng/ml)	373 (222, 627)	1060 (536, 2080)	2270 (1650, 3130)	3000 (1640, 5460)	4510 (2280, 8940)
C_{min} (ng/ml)	0.905 (0.527, 1.55)	3.16 (3.03, 3.29)	7.39 (4.10, 13.3)	7.91 (6.46, 9.68)	14.5 (6.46, 32.5)
C_{ave} (ng/ml)	55.5 (37.7, 81.7)	140 (107, 183)	338 (273, 418)	804 (703, 921) ^b	810 (587, 1120) ^c
AUC_{ss} (ng·hr/ml)	1332 (905.1, 1961)	3362 (2572, 4395)	8108 (6559, 10020)	19310 (16860, 22110) ^b	19430 (14100, 26780) ^c
CL_{ss}/F (ml/hr)	75100 (51000, 110000)	59500 (45500, 77800)	49300 (39900, 61000)	31100 (27100, 35600) ^b	41200 (29900, 56800) ^c

^a Median (Min – Max)

^b n=2, Subject 14 not included in calculation of summary statistics

^c n=4, Subject 22 not included in calculation of summary statistics

CT Scans of Patient 013 at Baseline (A) and after Cycle 24 (B)



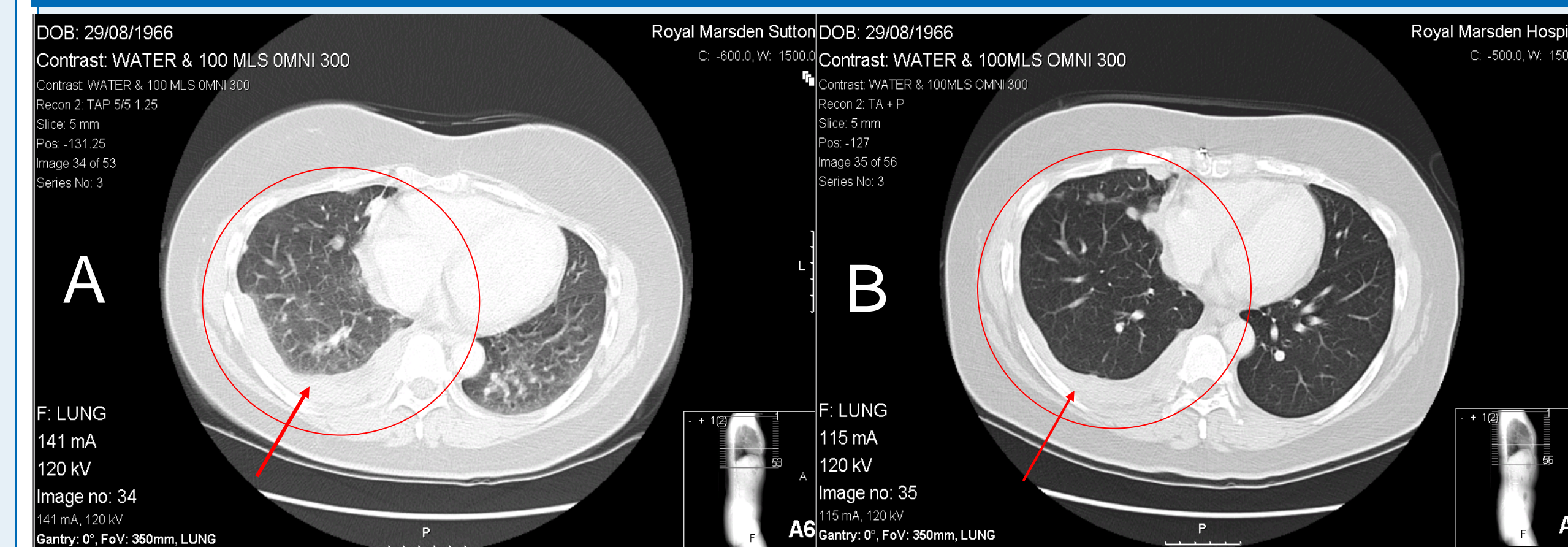
Female patient of 51 years with well differentiated retroperitoneal liposarcoma.

Scan A: Target lesions at baseline show below the diaphragm a large predominantly fat attenuation mass (24 cm in largest diameter) occupying most of the abdominal cavity.

Scan B: Target lesions after 24 treatment cycles (1 year) show no significant change in size (stable disease acc. to RECIST criteria).

In addition, multiple small pulmonary nodules suspicious for metastatic disease are unchanged (non-target lesions - data not shown).

CT Scans of Patient 015 at Baseline (A) and after Cycle 18 (B)



Female patient of 42 years with mediastinal thymoma (Masaoka Stage IV, extensive pleural based deposit).

Scan A: Target lesions at baseline include the tumour mass at the right base involving the diaphragmatic pleural surface and the right pulmonary horizontal fissure nodule.

Scan B: Target lesions after four treatment cycles show diminution in pleural thickening (stable disease acc. to RECIST criteria).

In addition, the conglomerate of nodular lesions in the right lung has reduced in size with a reduction in the number of nodules and less infiltrative changes (non-target lesions).

Conclusions

- Oral 4SC-201 could be safely administered in the tested dose ranges up to 800 mg on Day 1-5 in a 14-day cycle for several treatment cycles.
- Promising signs of efficacy were observed with 4SC-201 at dose levels of 400 mg, 600 mg and 800 mg once-daily dosing.
- The degree of HDAC enzyme inhibition was drug dose-dependent and ranged from 50% to 100% which was reached already at the 400 mg dose level or higher. Histone H4 acetylation level increased after dosing with a remarkable increase at the 800 mg dose level.
- A favorable PK profile of 4SC-201 in humans was observed with dose-dependent, high plasma exposure and low inter-subject variability indicating good bioavailability of the compound.
- Based on phase I results, the recommended phase II dosing of 4SC-201 is 600 mg once-daily as monotherapy.