Australian Government
Department of Health
Therapeutic Goods Administration

Nonclinical Evaluation Report

Selinexor [XPOVIO®]

Submission No: PM-2020-05458-1-6
Sponsor: Antengene (AUS) Pty Ltd

August 2021

TGA Health Safety Regulation

NONCLINICAL EVALUATION REPORT

Submission type: New chemical entity
Sponsor: Antengene (AUS) Pty Ltd
Generic name: **Selinexor**
Trade name: **XPOVIO®**
Dose form and strength: Film-coated tablet; 20 mg
Drug class: Exportin 1 inhibitor

Submission No: PM-2020-05458-1-6
Tox file No: E20-365471
TRIM reference: D20-3879244

Date authorised: 16 August 2021

Note: This evaluation report has been peer-reviewed and is authorised for release to the sponsor.

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SUMMARY, CONCLUSIONS AND RECOMMENDATION

- Antengene (Australia) Pty Ltd has applied to register a new chemical entity, selinexor (XPOVIO®), to be used for the treatment of multiple myeloma (in combination with bortezomib and dexamethasone), relapsed or refractory multiple myeloma (RRMM; in combination with dexamethasone), and relapsed or refractory diffuse large B-cell lymphoma (RR DLBCL) in adult patients.

- Recommended starting doses are 100 mg PO once weekly for multiple myeloma, 80 mg twice weekly for RRMM, and 60 mg twice weekly for RR DLBCL. Dosing is continued until disease progression or unacceptable toxicity.

- Module 4 was of overall high quality. The scope of the nonclinical dossier was adequate, consistent with the relevant TGA adopted guideline for the nonclinical evaluation of anticancer pharmaceuticals (ICH S9), although some minor deficiencies are noted. All pivotal safety-related studies were GLP-compliant except for one.

- Selinexor is the first in its pharmacological class: a selective inhibitor of nuclear export (SINE), targeting exportin-1 (XPO1). Binding of selinexor to XPO1 is covalent, but slowly reversible. Selinexor was shown to potently inhibit XPO1-mediated nuclear export *in vitro* (IC_{50}, ~20 nM), leading to accumulation of numerous tumour suppressor proteins and growth regulator proteins within the nucleus (their site of action) and suppression of the expression of several oncoproteins and translation/chaperone proteins. This induced cell-cycle arrest and cell death in cancer cell lines. Anti-tumour activity with selinexor treatment was demonstrated *in vivo* in mice bearing multiple myeloma, DLBCL and other tumour xenografts. Synergy in combination with dexamethasone and bortezomib (and various other agents) was shown. These studies offer support for the utility of selinexor in the proposed indications.

- Screening assays revealed no notable secondary pharmacological activity for selinexor.

- Safety pharmacology studies indicated no likely acute, pharmacologically-mediated effects of selinexor on CNS, cardiovascular or respiratory function.

- Rapid to moderately rapid absorption of selinexor after oral administration was seen in laboratory animal species and humans. Oral bioavailability was moderately high in mice, rats and monkeys. The plasma half-life was short in all species. Plasma protein binding was high in mice, rats, monkeys and humans. Rapid and wide tissue distribution of ^{14}C-selinexor-derived radioactivity was demonstrated in rats. Considerable penetration of the blood-brain barrier was evident in all species studies (mice, rats and monkeys). Metabolism of selinexor was limited, and involved CYP3A4, multiple UGTs and glutathione S-transferases. Excretion of selinexor and its metabolites was shown to be chiefly via the faeces/bile in rats.

- *In vitro* experiments examining the potential for pharmacokinetic drug interactions revealed inhibition of OATP1B3 by selinexor at clinically relevant concentrations. Clinically relevant inhibition of CYPs, UGTs and other transporters, and CYP induction, were not observed.

- Repeat-dose toxicity studies were performed with selinexor in rats and cynomolgus monkeys. All were conducted by the oral route. The pivotal studies were of 13 weeks duration and involved alternating three times and twice weekly dosing (*cf*. once or twice weekly dosing in patients). Shorter studies employed three times weekly dosing. The major targets for selinexor toxicity were the bone marrow, lymphoid tissues, gastrointestinal tract and male and female reproductive organs. Effects were typical of a cell-cycle inhibitor, and occurred at doses yielding systemic exposure levels below that of patients.

- Selinexor was negative in the standard battery of tests for genotoxicity. No carcinogenicity studies have been conducted; this is acceptable for a medicine indicated for the treatment of advanced cancer.
- Impairment of male and female fertility in patients is suggested by histopathological findings in the general repeat-dose toxicity program. Adverse effects on embryofetal development, including malformations and embryolethality, were observed with selinexor in rats and occurred at subclinical exposure levels. While strong concerns over embryofetal toxicity are held for selinexor, these are not so extreme as to warrant assignment to Category X as the Sponsor proposes. Assignment to Category D is recommended instead (consistent with the categorisation of similar agents).
- There are no nonclinical objections to the registration of XPOVIO® for the proposed indications provided a favourable risk/benefit balance is shown from clinical data.
- The draft Product Information should be amended as directed on pages 15–19.

ASSESSMENT

Antengene (AUS) Pty Ltd has applied to register a new chemical entity, selinexor (XPOVIO®). XPOVIO® is proposed to be used for the following indications:

- in combination with bortezomib and dexamethasone for the treatment of adult patients with multiple myeloma who have received at least one prior therapy;
- in combination with dexamethasone for the treatment of adult patients with relapsed or refractory multiple myeloma (RRMM) who have received at least three prior therapies and whose disease is refractory to at least one proteasome inhibitor, at least one immunomodulatory medicinal product (IMiD), and an anti-CD38 monoclonal antibody (mAb); and
- for the treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma (RR DLBCL), not otherwise specified, including DLBCL arising from follicular lymphoma, after at least 2 lines of systemic therapy.

XPOVIO® is presented as a film-coated tablet containing 20 mg selinexor. Recommended starting doses are 100 mg once weekly for multiple myeloma, 80 mg twice weekly for RRMM and 60 mg twice weekly for RR DLBCL. Dosing is to be continued until disease progression or unacceptable toxicity. The draft Product Information document provides details of dose modifications following the occurrence of adverse reactions.

General comments

Module 4 was of high quality and adequate in scope, consistent with the relevant TGA-adopted guideline for the nonclinical evaluation of anticancer pharmaceuticals (ICH S9). All pivotal safety-related studies were GLP-compliant except for one (on respiratory safety pharmacology, and which is only a minor deficiency).

Pharmacology

Molecular target and rationale

Selinexor is the first in its pharmacological class: a selective inhibitor of nuclear export (SINE), targeting exportin-1 (XPO1).

XPO1 is a major nuclear export protein, mediating the transport of >200 proteins and several classes of mRNA from the nucleus to the cytoplasm. XPO1 cargoes include many tumour suppressor proteins, and their export out of the nucleus (where they act) leads to their functional inactivation. Key proto-oncogenic mRNAs are also transported by XPO1, with cytoplasmic localisation required for their translation. Overexpression of XPO1 is seen in a large variety of malignancies — including MM and DLBCL (Luo *et al.*, 2018; Schmidt *et al.*, 2013) — and is associated with advanced disease, resistance to therapy and poor prognosis (see review by Camus *et al.*, 2017).

Primary pharmacology

In vitro, selinexor was shown to bind to the nuclear export signal (NES)-binding groove of XPO1, forming a covalent bond with Cys528. Although binding is covalent, it is slowly reversible. Concentration-dependent inhibition of XPO1-mediated nuclear export was demonstrated in experiments with human osteosarcoma cells, with high potency seen (IC_{50}, ~20 nM). Treatment with selinexor resulted in accumulation of numerous tumour suppressor proteins and growth regulator proteins (p53, p21, p27, FOXO1α, FOXO3α, IκB, APC, PP2Aα and survivin) within the nucleus in various human cancer cell lines; XPO1 inhibition was also shown to suppress expression of several oncoproteins (including c-Myc, Bcl2 and cyclin D1) and translation/chaperone proteins (Hsp70).

Selinexor induced cell-cycle arrest in both cancer and normal cells, but resultant cell death was highly specific to cancer cells (*e.g.*, the concentration of selinexor required to produce a 50% reduction in cell viability following 72 h exposure was 14 nM for the MM.1S multiple myeloma cell line *cf*. 22 μM for normal human dermal fibroblasts). Selinexor showed broad cytotoxic activity against a panel of multiple myeloma cell lines (median IC_{50}, 165 nM), as well as against other haematological (*e.g.*, AML, CLL and MCL) and solid tumour cells. In co-culture, the presence of bone marrow stromal cells did not affect the cytotoxic activity of selinexor towards multiple myeloma cells.

In vivo, significant anti-tumour activity with selinexor treatment was demonstrated in mice bearing multiple myeloma, DLBCL and other tumour xenografts, including cases of tumour regression. Enhanced anti-tumour activity was demonstrated with selinexor in combination with either dexamethasone and bortezomib, as well as with various other agents (carfilzomib, lenalidomide, panobinostat, venetoclax, bendamustine and etoposide). Of particular note, synergistic activity against proteasome inhibitor-resistant multiple myeloma was seen with selinexor in combination with bortezomib (*in vitro* and *in vivo*). Synergism with dexamethasone is likely to involve selinexor-induced glucocorticoid receptor accumulation in the nucleus (with nuclear export of the glucocorticoid receptor mediated by XPO1), and synergism with bortezomib seen to be through inhibition of NF-κB signalling by increasing IκBα in the nucleus.

The trans-isomer of selinexor (KPT-375) — the most common circulating human metabolite — possesses only limited pharmacological activity (~10% of its parent).

Secondary pharmacodynamics

No significant secondary activity was seen for selinexor in a suite of 112 receptor binding and enzymatic assays. The highest concentration tested (10 or 20 μM) is more than two orders of magnitude higher than the plasma C_{max} for unbound drug in patients at the maximum recommended clinical dose.

Safety pharmacology

Specialised safety pharmacology studies covered the CNS, cardiovascular and respiratory systems. The study examining respiratory effects was not conducted according to GLP (against recommendations in ICH S9 and ICH S7A), but the study was performed in an established laboratory and was well documented nevertheless. No effects on neurobehaviour were observed in rats following dosing at up to 50 mg/kg PO (estimated to yield ~11 times the clinical C_{max} at the maximum recommended clinical dose). Decreased body temperature was seen at 50 mg/kg PO, but was unaffected at 10 mg/kg (~6 times the clinical C_{max}). Selinexor was shown to be able to inhibit the hERG K^+ channel, but not at clinically relevant concentrations — the IC_{50} was 20.6 μM, >250 times higher than the peak plasma concentration of free selinexor expected in patients. ECG was unaffected in monkeys at ≤3 mg/kg PO in the general repeat-dose toxicity program (yielding a plasma C_{max} equivalent to that of patients at the maximum recommended clinical dose). Respiratory depression was observed with selinexor at ≥10 mg/kg PO in rats (~6 times the clinical C_{max}). No effect on respiratory function was observed at the next lowest dose, 2 mg/kg PO, but the plasma C_{max} in animals at this dose is only around half of the clinical C_{max}. Assessment of clinical relevance is hindered by the wide interval between exposure at the NOEL and LOEL, but given the margin of exposure at the dose required to affect respiration and considering the clinical signs observed in the general repeat-dose toxicity studies, an acute effect of selinexor to cause respiratory depression in patients seems unlikely.

Pharmacokinetics

Absorption of selinexor after oral administration was rapid or moderately rapid across species (T_{max} typically 0.5–1 h in mice and rats, ~1 h in dogs, ~2–4 h in cynomolgus monkeys and ~2 h in humans). Oral bioavailability was shown to be moderately high in mice, rats and monkeys (61–68%) [not determined in humans]. The plasma half-life was short (~3 h in rodents and dogs, ~4.5 h in monkeys and ~6 h in humans). Accordingly, repeat dosing (two or three times weekly) was associated with no significant accumulation. Exposure was generally dose-proportional. No sex-related differences were apparent.

Plasma protein binding of selinexor was high in mice, rats and cynomolgus monkeys (~95–97%), as in humans (95.1–95.4%), but only moderate in the dog (~54%). No preferential distribution into red blood cells was observed (examined in rats, monkeys and humans). The volume of distribution was high (>1 L/kg) across species. Consistent with this, wide tissue distribution of radioactivity was seen in (albino) rats after oral administration of ^{14}C-selinexor. Peak tissue levels were reached rapidly (1–2 h post-dose), and tissue concentrations generally declined in parallel with plasma. Outside of the GI tract, highest levels of radioactivity were observed in the kidney and liver (tissue:plasma C_{max} ratios, ~4–5.5). Considerable penetration of the blood-brain barrier was evident in rats, and in mice and monkeys too, with selinexor brain concentrations at 2 h post-dose found to be 61–72% of that for plasma. Binding to melanin was not investigated.

Metabolism of selinexor was limited both *in vitro* (in experiments with human liver microsomes, hepatocytes and S9 fraction) and *in vivo* (in rats, monkeys and humans). Experiments with recombinant human enzymes identified roles for CYP3A4 and multiple UGTs (most notably UGT1A1, 1A3 and 1A9) in metabolism. Metabolism of selinexor involved oxidation, hydrolysis, N-dealkylation, glucuronidation, and conjugation with glutathione and related moieties (cysteine, N-acteylcysteine and glycine-cysteine). Unchanged selinexor was by far the dominant circulating species in rats, monkeys and humans. The most common circulating metabolite in humans was the trans-isomer of selinexor (KPT-375), which was present at <5% of the peak level of the parent compound, and also formed in rats and monkeys (at similarly low levels). Excretion of selinexor and its metabolites was shown to be chiefly via the faeces/bile in rats. Urinary excretion appears to be similarly minor in monkeys and humans.

The key laboratory animal species used in the toxicity program — rats and cynomolgus monkeys — show sufficiently similar pharmacokinetic profiles to that in humans to support their use as appropriate models to investigate the toxicity of selinexor.

Pharmacokinetic drug interactions[1]

Selinexor produced no clinically relevant inhibition of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 or 3A4/5 or of UGT1A1, 1A3, 1A4, 1A6, 1A9 or 2B7 in experiments with human liver microsomes; the lowest IC_{50} values observed were more than 300 times greater than plasma C_{max} for unbound drug in patients at the maximum recommended human dose. The drug also produced no clinically relevant induction of CYP1A2, 2B6 or 3A4 in cultured human hepatocytes.

Selinexor was not a substrate of P-glycoprotein, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 or MATE2-K, nor of BCRP at clinically relevant concentrations. *In vitro* data indicate the potential for clinically relevant inhibition of OATP1B3, but not of other transporters examined (OATP1B1, OAT1, OAT3, OCT2, MATE1 and MATE2-K). Disappointingly, potential inhibition of

[1] Assessment is based on the EMA guideline on the investigation of drug interactions (CPMP/EWP/560/95/Rev.1 Corr.2), using values of 443.31 for molecular weight, 100 mg for dose, 693 ng/mL for clinical plasma C_{max}, 95.1% for plasma protein binding, 0.25 L for intestinal volume, 2.30 h^{-1} for absorption rate constant, 1 for fraction absorbed from gut to portal vein, and 97 L/h for total hepatic blood flow.

P-glycoprotein, BCRP and BSEP was not investigated (against recommendations in the relevant TGA-adopted EMA guideline).

Toxicity

Acute toxicity

A single-dose toxicity study was performed by the oral route in rats. No mortality was observed up to the highest dose tested (500 mg/kg), but animals were only observed to 24 h post-dose and this dose did produce substantial body weight loss. The GI tract was identified as a key target for toxicity. In the repeat-dose toxicity program, the highest dose levels where animals survived for at least 2 weeks were 5 mg/kg PO in rats and 3 mg/kg in monkeys.

Repeat-dose toxicity

Repeat-dose toxicity studies of up to 13 weeks duration were conducted with selinexor in rats and cynomolgus monkeys. All studies involved oral administration. Dosing frequency was similar to or greater than proposed clinically: alternating 2 and 3 times weekly dosing was used in the 13-week studies and three times weekly dosing in the shorter studies (*cf*. 1 or 2 times weekly administration in patients). The pivotal 13-week studies were adequately conducted in terms of the species used, duration, group size, dose selection and the monitoring and analyses performed.

Relative exposure

Exposure ratios have been calculated below based on animal:human plasma AUC adjusted for differences in dosing frequency (Table I). The highest dose levels tested in the pivotal studies yielded exposure below that of patients treated at the maximum recommended clinical dose. The higher doses employed in the 4-week studies yielded exposure in rats up to a modest multiple of that of patients, and exposure in monkeys only marginally above that of patients.

Major toxicities

The major targets for selinexor toxicity were the bone marrow and lymphoid tissues (accompanied by haematological changes), the gastrointestinal tract, and male and female reproductive organs. Numerous other tissues were also affected, including the adrenals, liver, pancreas, kidneys, bladder, salivary and other glands, heart, bone and brain.

Hypocellularity in the bone marrow and lymphoid depletion in the thymus, spleen and lymph nodes were observed at ≥1 mg/kg in the pivotal 13-week rat study. Additional changes in the bone marrow — single cell necrosis and haemorrhage — were seen in the shorter studies in rats (at ≥8.5/5 mg/kg for 4 weeks and at 50 mg/kg for 2 weeks). Monkeys showed bone marrow depletion with treatment at ≥1.5 mg/kg for 4 weeks and at 6 mg/kg for 2 weeks; lymphoid depletion was seen at all dose levels in the pivotal 13-week monkey study (≥0.1 mg/kg). Haematological effects observed in selinexor-treated animals consisted of decreases in white blood cell and red blood cell indices and platelets.

Body weight loss or decreased body weight gain, accompanied by decreased food consumption, was a common finding across the repeat-dose program. Clinical signs of gastrointestinal toxicity were observed in both rats (soft/mucoid faeces, diarrhoea) and monkeys (soft/loose faeces, red mucoid faeces, diarrhoea, emesis). Histopathological changes throughout the GI tract were a particularly prominent feature of the 4-week study in rats, with findings of mucosal atrophy and single cell necrosis in the stomach and small and large intestines, and stomach submucosal oedema and inflammation, encountered at ≥8.5/5 mg/kg, and erosion and ulceration of the stomach and intestinal crypt hyperplasia at 15 mg/kg. Microscopic changes in the GI tract in monkeys were widespread with treatment at 6 mg/kg for 2 weeks (mucosal atrophy of the stomach and small and large intestines, and mucosal ulceration in the stomach and caecum), but were limited in the 4-week study (mucosal atrophy in the ileum at 3 mg/kg) and absent in the 13-week study (<1 mg/kg).

Damage to the male reproductive system was seen at all dose levels in the pivotal 13-week rat study (≥0.25 mg/kg, associated with exposure ~26 times lower than in patients at the maximum recommended clinical dose). Findings comprised germ cell degeneration, spermatid retention and tubular degeneration in the testes, decreased luminal sperm in the epididymides, and decreased secretion in the seminal vesicles. With treatment at 4 mg/kg (relative exposure, 0.61), severe tubular degeneration occurred in both testes in every animal, and was not reversed after a 4-week treatment-free period. The testicular effects are consistent with Sertoli cell damage, which could result in permanent loss of spermatogenesis. Dosing at ≥8.5/5 mg/kg in the 4-week rat study revealed additional effects on male reproductive tissues: glandular epithelial atrophy, decreased secretion and single cell necrosis in the prostate, coagulating gland and seminal vesicles. In monkeys, single cell necrosis was observed in the testes with treatment at 6 mg/kg for 2 weeks and at ≥1.5 mg/kg for 4 weeks.

Oestrus cycling stopped in female rats treated with selinexor at 15 mg/kg for 4 weeks (accompanied by uterine atrophy) and was disrupted at 4 mg/kg in the pivotal 13-week study. Decreased ovarian follicles were observed at ≥2 mg/kg in the 4-week study. Findings of increased corpora lutea (at ≥1 mg/kg) and increased vaginal mucification (at 4 mg/kg) in the 13-week study are consistent with repetitive pseudopregnancy or pseudopregnancy-like state; and increased uterine weight and incidence of pre-oestrus (seen at 4 mg/kg) suggest a possible-estrogenic effect of selinexor. Metaphysis hyperostosis of the femur, observed in female rats at ≥1 mg/kg in the 13-week study, might have occurred as a consequence of hormonal perturbation. Effects on female reproductive tissues were not seen in monkeys.

In the adrenal, cortical hypertrophy was observed at ≥1 mg/kg in rats and at 1 mg/kg in monkeys in the 13-week studies (as a response to stress), and single cell necrosis (≥8.5/5 mg/kg) and necrosis (15 mg/kg) were seen in the 4-week rat study. Microscopic changes in the liver were limited to female rats in the 13-week study, comprising centrilobular hepatocellular hypertrophy (at all dose levels; ≥0.25 mg/kg) and increased mitoses (at 4 mg/kg).

Pancreatic single cell necrosis was observed in rats with treatment at ≥8.5/5 mg/kg for 4 weeks; and pancreatic necrosis (≥1.5 mg/kg) and acinar atrophy (3 mg/kg) were reported in the 4-week monkey study. Pancreatic lesions were accompanied by elevated serum amylase and lipase levels. Kidney lesions were observed in rats with treatment at 15 mg/kg for 4 weeks (glomerulopathy, tubular degeneration, haemoglobin casts and hyaline droplets), and in monkeys at ≥2.5 mg/kg for 2 weeks (multifocal necrosis) and at 3 mg/kg for 4 weeks (nephrosis). Single cell necrosis was frequently observed in the urinary bladder of rats treated at 15 mg/kg in the 4-week study.

Atrophy/necrosis of the salivary gland occurred at ≥8.5/5 mg/kg in rats and at ≥1.5 mg/kg in monkeys in the 4-week studies. The Harderian and lacrimal glands also showed atrophy and single cell necrosis, and there was atrophy of the pituitary, in rats in the 4-week study.

Cardiomyocyte degeneration and necrosis, and decreased cortical and/or trabecular bone in the femur and sternum, were observed in rats treated at 15 mg/kg for 4 weeks. There were no similar findings in any of the monkey studies.

CNS lesions were only observed in the 2-week studies and at lethal doses, with findings of necrosis of granule cells of the cerebellum in rats at 50 mg/kg and in monkeys at 6 mg/kg.

Effects on the pancreas, kidney, bladder, salivary and other glands and the heart were not observed in the 13-week studies in rats and monkeys, and effects on the brain were not observed in either the 4- or 13-week studies in the two species, indicating that these tissues are not among the most sensitive to selinexor toxicity.

Reversibility was demonstrated except for effects on male and female reproductive tissues.

Genotoxicity

The potential genotoxicity of selinexor was investigated in the standard battery of tests — a bacterial reverse mutation assay, an *in vitro* assay for clastogenicity (using human lymphocytes), and a bone marrow micronucleus test (in rats). The conduct of the studies was in accordance with ICH guidelines. Concentrations/doses were appropriate (up to maximum recommended levels or limited by cytotoxicity), a suitable set of *S. typhimurium* and *E. coli* strains was used in the bacterial gene mutation assay, and the assays were appropriately validated. Negative results were returned for selinexor in all assays.

Carcinogenicity

No studies were submitted. This is acceptable under ICH S9 and ICH S1A for an agent for the treatment of advanced cancer.

Reproductive and developmental toxicity

Reproductive and developmental toxicity studies submitted by the Sponsor covered embryofetal development only. The absence of studies covering other stages (fertility, early embryonic development, and pre-/postnatal development) is acceptable under ICH S9 based on the indication. Only a single species (rat) was used in the embryofetal development program, with a study in a second non-rodent species not warranted given the adverse findings. Systemic exposure in the definitive embryofetal development study was well below that of patients (Table II).

No data on placental transfer or excretion in milk were included in the submission.

s47 [redacted]

Impairment of male fertility is expected and impairment of female fertility is possible in patients treated with XPOVIO® based on histopathological findings in reproductive tissues in the general repeat-dose toxicity program (see above).

Total litter loss was observed with dosing at 5 mg/kg/day PO in a pilot study in rats (expected to have yielded 0.6 times the clinical exposure to selinexor). In the main embryofetal development study, treatment with selinexor was associated with decreased fetal weight, inhibition of ossification and increased skeletal variations at ≥0.75 mg/kg/day and fetal malformations (fetal oedema, microphthalmia, malpositioned kidney and persistent truncus arteriosus) at 2 mg/kg/day.

Pregnancy classification

The sponsor has proposed Pregnancy Category X. While strong concerns for the potential for embryofetal harm are held for selinexor, these are not so extreme as to warrant assignment to Category X. The categorisation proposed by the Sponsor is also at odds with the absence of known or suspected pregnancy as a contraindication in the proposed Product Information document. Category D is recommended instead (with this being the typical category for existing registered cell cycle inhibitors, producing similar findings in animals).

Paediatric use

Selinexor is not proposed for paediatric use and no specific studies in juvenile animals were submitted.

Local tolerance

Selinexor was shown not to be an ocular irritant (*in vitro* bovine cornea assay). Mild dermal contact hypersensitivity was seen with selinexor in the guinea-pig skin sensitisation test.

Immunotoxicity

Specialised immunotoxicity studies were not conducted with selinexor. Immunosuppression is expected based on effects on lymphoid organs observed in the general repeat-dose toxicity studies.

Phototoxicity

Selinexor absorbs UV light. The drug was shown to not be phototoxic in an adequately conducted *in vitro* assay using Balb/c 3T3 mouse fibroblast cells.

Impurities

The proposed drug substance and drug product specifications are considered to be toxicologically acceptable (see pages 61–63 for further discussion).

Comments on the Nonclinical Safety Specification of the Risk Management Plan

Key safety concerns arising from nonclinical data are adequately identified in the Sponsor's draft Risk Management Plan (Part II, Module SII) except with regard to embryofetal harm. The Sponsor only notes findings of decreased fetal weight and increased skeletal variations (denoting a developmental delay). Selinexor is also seen to be embryolethal (universally at 5 mg/kg/day) and to produce malformations (at 2 mg/kg/day) in rats. These doses are associated with systemic exposure levels below that of patients.

PRODUCT INFORMATION

The following comments refer to the draft Product Information accompanying the Sponsor's S.31 response (eCTD sequence 0003). Where changes are suggested (highlighted in yellow below), text proposed to be inserted is underlined and text to be deleted is shown struck-through.

4.4 SPECIAL WARNING AND PRECAUTIONS FOR USE

A section headed "Embryo-Feotal [sic] Toxicity" should be deleted. Relevant information should be presented in Section 4.6 (Fertility, pregnancy and lactation) instead. Thus:

> ~~**Embryo-Feotal Toxicity**~~
>
> ~~Women of childbearing potential should be advised to avoid becoming pregnant or abstain from sexual intercourse while being treated with selinexor and for at least 1 week following the last dose of XPOVIO (see Section 4.6 FERTILITY, PREGNANCY AND LACTATION).~~
>
> ~~Male patients of reproductive potential should be advised to use effective contraceptive measures or abstain from sexual activity to prevent pregnancy during treatment with XPOVIO and for at least 1 week following the last dose of XPOVIO (see Section 4.6 FERTILITY, PREGNANCY AND LACTATION).~~

4.5 INTERACTIONS WITH OTHER MEDICINES AND OTHER FORMS OF INTERACTIONS

Findings from relevant *in vitro* studies should be reported here. Thus:

> Exposure of XPOVIO was not affected by co-administration with paracetamol at a daily dose up to 1000 mg.
>
> Selinexor is a substrate of CYP3A4. Concomitant use of strong CYP3A4 inducer might lead to lower exposure of XPOVIO.
>
> *In vitro*, selinexor was shown to not inhibit CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 or 3A4/5, or UGT1A1, 1A3, 1A4, 1A6, 1A9 or 2B7, at clinically relevant concentrations. Selinexor is not an inducer of CYP1A2, 2B6 or 3A4, and is not a substrate of P-glycoprotein, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 or MATE2-K. Selinexor inhibits OATP1B3, but not OATP1B1, OAT1, OAT3, OCT2, MATE1 or MATE2-K, at clinically relevant concentrations.

4.6 FERTILITY, PREGNANCY AND LACTATION

Effects on fertility

Relevant findings from animal studies should be described here rather than in Section 5.3 (Preclinical safety data). Effects on the male reproductive tract occurred at ≥0.25 mg/kg in the 13-week rat study, rather than at ≥1 mg/kg as reported. Given the subclinical exposure, animal:human exposure is better described qualitatively. Text here should only relate to the potential for impairment of fertility; a separate section on contraceptive use should follow. Thus:

> **Effects on fertility**
>
> Based on findings in animals, XPOVIO may impair fertility in females and males. ~~Women of childbearing potential should be advised to avoid becoming pregnant or abstain from sexual intercourse while being treated with XPOVIO and for at least 1 week following the last dose of XPOVIO. A pregnancy test is recommended for women of childbearing potential prior to initiating XPOVIO treatment.~~

~~Male patients of reproductive potential should be advised to use effective contraceptive measures or abstain from sexual activity to prevent pregnancy during treatment with XPOVIO and for at least 1 week following the last dose of XPOVIO.~~

No fertility studies have been conducted with selinexor in animals. Impairment of male and female fertility in patients is suggested by findings in general repeat-dose toxicity studies. Selinexor reduced sperm, spermatids and germ cells in the epididymides and testes in rats at oral doses ≥0.25 mg/kg, decreased ovarian follicles in rats at ≥2 mg/kg, and produced single cell necrosis in the testes of monkeys with treatment at ≥1.5 mg/kg. These doses resulted in systemic exposure (plasma AUC) well below that of patients at the maximum recommended human dose. Reversibility of the male reproductive tract findings was not demonstrated in animals.

Women of childbearing potential / Contraception in males and females

Women of childbearing potential should be advised to avoid becoming pregnant or abstain from sexual intercourse while being treated with XPOVIO and for at least 1 week following the last dose of XPOVIO. A pregnancy test is recommended for women of childbearing potential prior to initiating XPOVIO treatment.

Male patients of reproductive potential should be advised to use effective contraceptive measures or abstain from sexual activity to prevent pregnancy during treatment with XPOVIO and for at least 1 week following the last dose of XPOVIO.

Use in pregnancy

The Sponsor proposes Pregnancy Category X and the following statement:

> *"There are no data from the use of XPOVIO in pregnant women. Based on findings in animal studies and its mechanism of action (see Section 5.3 PRECLINICAL SAFETY DATA), XPOVIO can cause foetal harm when administered to a pregnant woman. Selinexor administration to pregnant animals during organogenesis resulted in structural abnormalities and alterations to growth at exposures below those occurring clinically at the recommended dose.*
>
> *There are no available data in pregnant women to inform the drug-associated risk.*
>
> *XPOVIO is not recommended during pregnancy and in women of childbearing potential not using contraception. Verify the pregnancy status of females of reproductive potential prior to initiating XPOVIO.*
>
> *Advise pregnant women of the potential risk to a foetus. If the patient becomes pregnant while taking XPOVIO, treatment should be immediately discontinued, and the patient should be apprised of the potential hazard to the foetus."*

As discussed in the Assessment, although serious concern for potential embryofetal harm is held, Pregnancy Category X is not warranted (and is also inconsistent with known or suspected pregnancy not being included as a contraindication in Section 4.3 and the strength of the warning against use in pregnancy [*i.e.*, "not recommended" vs. "must not be used"]). **Pregnancy Category D** should be used instead. Relevant animal findings should be described here rather than in Section 5.3 (Preclinical safety data). The preferred Australian spelling of *fetus / fetal* should be used, the additional 'o' having no etymological basis[2]. Thus:

[2] Macquarie Dictionary usage note: The etymology of this word is from a Latin form *fetus*. The spelling *foetus*, probably based on false analogy with words such as *oedema* and *oestrogen*, was widely used, although health authorities increasingly recommend the spellings *fetus* and *fetal*.

> **Use in pregnancy — Category X**
>
> There are no data from the use of XPOVIO in pregnant women. Based on findings in animal studies and its mechanism of action ~~(see Section 5.3 PRECLINICAL SAFETY DATA)~~, XPOVIO can cause ~~foetal~~ fetal harm when administered to a pregnant woman. ~~Selinexor administration to pregnant animals during organogenesis resulted in structural abnormalities and alterations to growth at exposures below those occurring clinically at the recommended dose. There are no available data in pregnant women to inform the drug-associated risk.~~
>
> Administration of selinexor to pregnant rats during organogenesis resulted in reduced fetal weight, impaired ossification and increased fetal skeletal variations at oral doses ≥0.75 mg/kg/day. Malformations (microphthalmia, fetal oedema, malpositioned kidney and persistent truncus arteriosus) were observed at 2 mg/kg/day. These doses yield systemic exposure well below that of patients at the maximum recommended clinical dose (4–16 times lower than the human AUC at 100 mg). At 5 mg/kg/day (estimated to yield 0.6 times the clinical AUC), selinexor was embryolethal in rats.
>
> XPOVIO is not recommended during pregnancy and in women of childbearing potential not using contraception. Verify the pregnancy status of females of reproductive potential prior to initiating XPOVIO.
>
> Advise pregnant women of the potential risk to a ~~foetus~~ fetus. If the patient becomes pregnant while taking XPOVIO, treatment should be immediately discontinued, and the patient should be apprised of the potential hazard to the ~~foetus~~ fetus.

Use in lactation

The proposed text — reproduced below — is considered to be acceptable:

> It is unknown whether XPOVIO or its metabolites are excreted in human milk, or their effects on the breastfed child or milk production. A risk to breast-fed children cannot be excluded. Breast-feeding should be discontinued during treatment with XPOVIO and for 1 week after the last dose.

5.1 PHARMACODYNAMIC PROPERTIES

Mechanism of action

Nuclear export is erroneously ascribed to selinexor rather than XPO1. The set of proteins described as tumour suppressor proteins additionally includes growth regulator proteins, and the set of cited oncoproteins additionally includes a translation/chaperone protein. Data on suppression of oncogenic mediators and translation/chaperone proteins was for another XPO1 inhibitor and not selinexor, and did not include Bcl-6 (Tabe *et al.*, 2015). *In vitro* and *in vivo* are recommended to appear in italics. The statement should be modified as follows:

> Selinexor is a reversible covalent selective inhibitor of nuclear export (SINE) compound that specifically blocks exportin 1 (XPO1). XPO1 is a major nuclear export protein that transports cargo proteins and several classes of mRNA from the nucleus to the cytoplasm. ~~Selinexor is the major mediator of the nuclear export of many cargos protein including~~ XPO1 cargoes include many tumour suppressor proteins (TSPs), growth regulator~~s~~ proteins (GRPs) and mRNAs of growth promoting (oncogenic) proteins. XPO1 inhibition by selinexor leads to marked accumulation of TSPs and GRPs (such as p53, p21, FOXO and IκB) in the nucleus ~~such as p53, p21, FOXO and IkB~~ (their site of action), ~~reductions in~~ and reduced expression of several oncoproteins (such as c-Myc, ~~HSp70,~~ Bcl2 ~~and Bcl6~~ and

> cyclin D1) and translation/chaperone proteins (Hsp70), resulting in cell cycle arrest and apoptosis of cancer cells. The combination of selinexor and dexamethasone or bortezomib demonstrated synergistic cytostatic and cytotoxic effects in multiple myeloma *in vitro* and *in vivo* models, including those resistant to proteasome inhibitors. Selinexor demonstrated pro-apoptotic activity *in vitro* in multiple myeloma and diffuse large B-cell lymphoma cell lines, in murine xenograft models as well as in patient tumour samples.

5.2 PHARMACOKINETIC PROPERTIES

Distribution

Mean plasma protein binding by selinexor was 95.4% at 1 µM and 95.1% at 10 µM (Study KS-50013). Thus, with appropriate rounding:

> Selinexor is ~~95.0%~~ 95% bound to human plasma proteins. In a population pharmacokinetic (PK) analysis, the apparent volume of distribution (Vd/F) of selinexor was 133 L in cancer patients.

Metabolism

The proposed statement is supported by nonclinical data. Preferred Australian spelling should be used and a full-stop added. Thus:

> Selinexor is ~~metabolized~~ metabolised by CYP3A4, multiple UDP-glucuronosyltransferases (UGTs) and glutathione S-transferases (GSTs).

5.3 PRECLINICAL SAFETY DATA

In accordance with the form for providing Product Information approved by the Secretary under subs. 7D(1) of the Therapeutic Goods Act 1989, this section should only contain information pertaining to genotoxicity and carcinogenicity. Information from animal studies relating to reproductive and developmental toxicity is to be presented in Section 4.6 (Fertility, pregnancy and lactation) and appropriate text to use is covered above. The following non-standard section should be deleted (and not relocated):

> ~~**Reproductive toxicity**~~
>
> ~~In animal reproduction studies, administration of selinexor to pregnant rats during organogenesis resulted in structural abnormalities and alterations to growth at exposures that were below those occurring clinically at the recommended dose. In other short-term toxicology studies, effects were observed in male and female reproductive organs in monkeys and developmental effects were seen with daily exposure in rats~~
>
> ~~Fertility studies in animals have not been conducted with selinexor. In repeat-dose oral toxicity studies, selinexor was administered for up to 13 weeks in rats and monkeys. Reduced sperm, spermatids, and germ cells in epididymides and testes were observed in rats at ≥1 mg/kg, decreased ovarian follicles were observed in rats at ≥2 mg/kg, and single cell necrosis of testes was observed in monkeys at ≥1.5 mg/kg. These dose levels resulted in systemic exposures approximately 0.11, 0.28, and 0.53 times, respectively, the exposure (AUC_{last}) in humans at the recommended human dose of 80 mg.~~
>
> ~~In an embryo-fetal development study in pregnant rats, daily oral administration of selinexor at 0, 0.25, 0.75, or 2 mg/kg throughout organogenesis caused incomplete or delayed ossification, skeletal variations, and reduced fetal weight compared with controls~~

> ~~at a dose of 0.75 mg/kg (approximately 0.08-fold of human area under the curve [AUC] at the recommended dose). Malformations were observed at 2 mg/kg, including microphthalmia, fetal edema, malpositioned kidney, and persistent truncus arteriosus.~~

Genotoxicity

Minor changes to the description of the assay in human lymphocytes and use of italics are recommended:

> Selinexor was not mutagenic *in vitro* in a bacterial reverse mutation (Ames) assay and was not clastogenic in either the *in vitro* ~~cytogenetic~~ chromosomal aberration assay in human lymphocytes or in the *in vivo* rat micronucleus assay.

Carcinogenicity

The proposed statement — reproduced below — is acceptable:

> Carcinogenicity studies have not been conducted with selinexor.

MAIN BODY OF REPORT

1. INTRODUCTION

1.1. BACKGROUND

Antengene (AUS) Pty Ltd has applied to register a new chemical entity, selinexor (XPOVIO®). XPOVIO® is proposed to be used for the following indications:

- in combination with bortezomib and dexamethasone for the treatment of adult patients with multiple myeloma who have received at least one prior therapy;
- in combination with dexamethasone for the treatment of adult patients with relapsed or refractory multiple myeloma (RRMM) who have received at least three prior therapies and whose disease is refractory to at least one proteasome inhibitor, at least one immunomodulatory medicinal product (IMiD), and an anti-CD38 monoclonal antibody (mAb); and
- for the treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma (RR DLBCL), not otherwise specified, including DLBCL arising from follicular lymphoma, after at least 2 lines of systemic therapy.

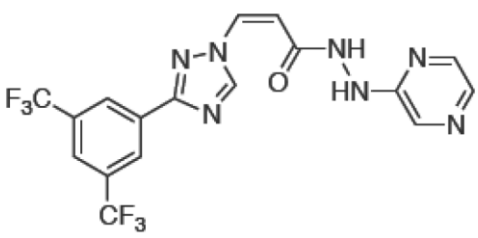
Proposed starting dose regimens are shown in Table 1.1. Dosing is continued until disease progression or unacceptable toxicity. The draft Product Information document provides details of dose modifications following the occurrence of adverse reactions.

Table 1.1. Proposed starting dosing regimens

Disease	Starting dose regimen
Multiple myeloma (MM)	*on a 35-day cycle…* • selinexor: 100 mg PO once weekly on days 1, 8, 15, 22 & 29 • bortezomib: 1.3 mg/m^2 SC once weekly on days 1, 8, 15 & 22 • dexamethasone: 20 mg PO on days 1, 2, 8, 9, 15, 16, 22, 23, 29 & 30
Relapsed or refractory multiple myeloma (RRMM)	• selinexor: 80 mg PO on days 1 & 3 of each week • dexamethasone: 20 mg PO on days 1 & 3 of each week
Relapsed or refractory diffuse large B-cell lymphoma (RR DLBCL)	• selinexor: 60 mg PO on days 1 & 3 of each week

The maximum recommended dose of selinexor on any occasion is 100 mg PO, and 160 mg PO over a week.

1.2. CHEMISTRY AND FORMULATION



Selinexor
(2*Z*)-3-{3-[3,5-bis(trifluoromethyl)phenyl]-1*H*-1,2,4-triazol-1-yl}-N′-(pyrazin-2-yl)prop-2-enehydrazide
Molecular formula: $C_{17}H_{11}F_6N_7O$
Molecular weight: 443.31
CAS No.: 1393477-72-9
pK_a: 10.20 ± 0.07 (in water, spectrophotometric at 256 nm); 10.05 ± 0.05 (in water, spectrophotometric at 350 nm);
Log P: 3.98
Laboratory code: KPT-330; KH8; Karazine St4

XPOVIO® is presented as a film-coated tablet, formulated as follows:

s47

[redacted]

1.3. RELATIONSHIP TO OTHER DRUGS

Selinexor is the first in its pharmacological class (selective inhibitor of nuclear export [SINE]; exportin-1 inhibitor).

1.4. INTERNATIONAL REGULATORY STATUS

At the time of submission, the US FDA had approved similar applications for the treatment of RRMM (July 2019) and RL DLBCL (June 2020), and had received an application to extend the indications to include MM (submitted in May 2020 [subsequently approved in December 2020). An application to the EMA for use in refractory multiple myeloma was under evaluation at the time of submission (submitted in January 2019 [subsequently approved in March 2021]).

2. PRIMARY PHARMACOLOGY

Selinexor is an inhibitor or exportin 1 (XPO1) [also called chromosomal regional maintenance 1 (CRM1)].

XPO1 is a major nuclear export protein, mediating the transport of >200 proteins and several classes of mRNA from the nucleus to the cytoplasm. XPO1 cargoes include many tumour suppressor proteins, and their export out of the nucleus (where they act) leads to their functional inactivation. Key proto-oncogenic mRNAs are also transported by XPO1, with cytoplasmic localisation required for their translation.

2.1. *IN VITRO* STUDIES

In vitro experiments with selinexor are summarised in Table 2.1. Some further information from selected literature publications is also reported below.

Table 2.1. Summary of *in vitro* studies

Study & experimental details	Major findings
Study KPT-00005 *Binding of selinexor to XPO1;* *Inhibition of XPO1/cargo protein interactions* Crystal structure of selinexor bound to *Saccharomyces cerevisiae* XPO1 in complex with human Ran-GppNHp & *S. cerevisiae* RanBP1 Incubation of purified recombinant XPO1 with immobilised nuclear export signal (NES)-containing cargo proteins, followed by removal of unbound proteins by washing and remaining bound proteins separated by SDS-PAGE & visualised by Coomassie Blue staining	• selinexor binds in the nuclear export signal (NES)-binding groove of XPO1 and forms a covalent bond with Cys539 (equivalent to Cys528 in human XPO1) • selinexor (5 μM) inhibited the association of XPO1 with three different classical NESs/cargos (PKI-NES, MVM-NS2-NES & full-length snurportin); maximal inhibition of XPO1 binding to PKI-NES was observed following 60 min of pre-incubation with selinexor • dialysis for 24 h restored XPO1/PKI-NES association to 36% of the vehicle control [= interaction of selinexor with XPO1 is slowly reversible]
Study KS-01 (KS-0001) *Inhibition of XPO1-mediated transport* U2OS [human osteosarcoma] cells stably expressing a green fluorescent protein (GFP)-tagged HIV-Rev protein fused to the cAMP-dependent Protein Kinase Inhibitor (PKI) nuclear export signal (Rev-GFP); 4 h treatment at 0.17–10 μM; nuclear/cytoplasmic localisation determined by fluorescence microscopy	• selinexor inhibited XPO1-mediated nuclear export of Rev-GFP in a concentration-dependent manner (IC_{50}, ~20 nM), increasing nuclear Rev-GFP by up to ~4-fold
Study KS-02 (KS-0002) *Inhibition of wildtype & mutant XPO1-mediated transport* U2OS cells stably expressing Rev-GFP transiently transfected with constructs expressing wildtype human XPO1 or a Cys528Ser mutant; 4 h treatment at 30 μM; nuclear/cytoplasmic localisation determined by fluorescence microscopy	• selinexor inhibited nuclear export of Rev-GFP in cells expressing wildtype XPO1, but not cells expressing the Cys528Ser mutant form [= selinexor targets the Cys528 residue of XPO1]
Study KS-04 (KS-0004) *Recovery of inhibition of XPO1-mediated transport* U2OS cells stably expressing Rev-GFP; 4 h treatment at 0.17 nM to 10 μM, followed by recovery in fresh media for up to 28 h; nuclear/cytoplasmic localisation determined by fluorescence microscopy	• restoration of cytoplasmic localisation of Rev-GFP occurred in a time- & concentration-dependent manner (*e.g.*, 50% recovery at 12, 16 & 24 h following exposure to selinexor at 126, 150 & 344 nM for 4 h) • exposure to ~0.5–1 μM selinexor for 4 h was sufficient to maintain at least 50% inhibition of XPO1-mediated transport for at least 28 h following removal of the drug; cells treated with 1.1–10 μM selinexor showed no recovery after 28 h
Study KS-03 (KS-0003) *Nuclear retention of tumour suppressor proteins & growth regulatory proteins in human cancer cell lines* A549 [lung carcinoma], H226 [lung squamous cell carcinoma] & HCT116 [colorectal carcinoma] cells; 4 or 24 h treatment at 1 μM; nuclear/cytoplasmic localisation of known XPO1 cargo proteins determined by immunofluorescence microscopy	• selinexor ↑ nuclear localisation of: APC *[HCT116 cells]* FOXO1α FOXO3α IκB p21 *[H226 cells]* p27 PP2Aα survivin p53 *[A549 cells]*

cont.

Table 2.1. Summary of *in vitro* studies (cont.)

<table>
<tr><th>Study & experimental details</th><th>Major findings</th></tr>
<tr><td>Tabe et al. (2015)
Effect on oncoprotein & translation/ chaperone mRNA/protein expression
MCL cell lines Z138, JVM2, MINO & Jeko-1; treatment with KPT-185 [not selinexor] at 50–500 nM for 18 h followed by gene expression analysis using real-time PCR & protein expression by immunoblotting</td><td>• another XPO1 inhibitor, KPT-185, produced suppression of oncogenic mediators (XPO1, cyclin D1, c-Myc, PIM1 & Bcl-2) & downregulation of translation/chaperone proteins (PIM2, EEF1A1, EEF2 & Hsp70)</td></tr>
<tr><td>Study KS-05 (KS-0005)
Effect on the cell cycle & cell viability in human cell lines
HCT116 [colorectal carcinoma] with wildtype or null p53, Jurkat [acute T cell leukaemia], MM.1S [multiple myeloma] & normal human dermal fibroblast cells; 72 h treatment at 0.51 nM to 30 μM; cell viability determined by luminescent detection of ATP and DNA synthesis & cell cycle distribution determined by BrdU incorporation</td><td>• selinexor ↓ cell viability in all of the cancer cell lines; absence of p53 was associated with ↓ sensitivity to selinexor-induced cell death
• IC_{50} values for inhibition of cell viability:
— HCT116 with wildtype p53: 148 nM
— HCT116 with p53 null: 1.17 μM
— Jurkat: 17.8 nM
— MM.1S: 14 nM
— normal human dermal fibroblasts: 22 μM
• no cytotoxicity to normal human dermal fibroblasts at up to 10 μM
• selinexor (0.5 or 1 μM) induced cell-cycle arrest & inhibited or abolished DNA synthesis in all cell lines (including normal human dermal fibroblasts) within one day of treatment
• blockade of DNA synthesis happened faster & more efficiently in HCT116 cells with wildtype p53 cf. p53 null cells</td></tr>
<tr><td>Study KS-07 (KS-0007)
Cytotoxicity to human cancer cell lines
Panel of 12 multiple myeloma & 8 non-myeloma cell lines; 72 h treatment at 15.6 nM to 1 μM; MTT assay for cell viability</td><td>• selinexor produced concentration-dependent ↓ in cell viability
• multiple myeloma cell lines showed greater sensitivity (IC_{50} values, 20–434 nM & 60–100% cell killing) cf. non-MM cell lines (IC_{50} values, >500 nM & 20–60% cell killing) other than Jurkat cells
<table>
<tr><th colspan="4">IC_{50} values for inhibition of cell viability</th></tr>
<tr><th colspan="2">multiple myeloma cell lines</th><th colspan="2">non-MM cell lines</th></tr>
<tr><td>KMS11</td><td>93.4 nM</td><td colspan="2">transformed cells:</td></tr>
<tr><td>KMS2PE</td><td>184 nM</td><td>293</td><td>1047 nM</td></tr>
<tr><td>KMS18</td><td>149 nM</td><td>COS</td><td>552 nM</td></tr>
<tr><td>OPM1</td><td>165 nM</td><td>CHO</td><td>1329 nM</td></tr>
<tr><td>H929</td><td>59.9 nM</td><td colspan="2">solid-tumour derived:</td></tr>
<tr><td>JJN3</td><td>372 nM</td><td>A549</td><td>2953 nM</td></tr>
<tr><td>U266</td><td>164 nM</td><td>MCF7</td><td>1254 nM</td></tr>
<tr><td>8226</td><td>434 nM</td><td>SKMEL28</td><td>930 nM</td></tr>
<tr><td>MY5</td><td>20.1 nM</td><td colspan="2">Burkitt's lymphoma:</td></tr>
<tr><td>SKMM2</td><td>333 nM</td><td>Raji</td><td>1583 nM</td></tr>
<tr><td>MM.1S</td><td>21.3 nM</td><td colspan="2">T-cell leukaemia:</td></tr>
<tr><td>OPM2</td><td>175 nM</td><td>Jurkat</td><td>4.05 nM</td></tr>
</table></td></tr>
<tr><td>Study KS-08 (KS-0008)
Cytotoxicity to MM cell lines in the absence & presence of bone marrow stromal cells
Panel of 11 human multiple myeloma cell lines ± bone marrow stromal cells; 48 or 60 h treatment at 10 nM to 1 μM; cell viability determined by luminescent detection of ATP</td><td>• selinexor induced cytotoxicity of multiple myeloma cell lines both in the absence & presence of bone marrow stromal cells; IC_{50} values were generally ≤250 nM and unchanged in the presence of bone marrow stromal cells
• selinexor had very little effect on the viability of human bone marrow stromal cells</td></tr>
</table>

cont.

Table 2.1. Summary of *in vitro* studies (cont.)

<table>
<tr><th>Study & experimental details</th><th>Major findings</th></tr>
<tr><td>Study KS-09 (KS-0009)
Cytotoxicity to human chronic lymphocytic leukaemia (CLL) in the absence & presence of stromal cells
CD19+ cells from four CLL patients ± co-culture with HS-5 [human fibroblast] cells; 48 h treatment at 2.5 µM; MTS assay for cell viability</td><td>• treatment with selinexor resulted in 25–60% cell killing at 72 h
• cytotoxicity was unaffected by the presence of stromal cells</td></tr>
<tr><td>Study KS-10 (KS-0010)
Cytotoxicity to acute myeloid leukaemia (AML) & acute lymphoblastic leukaemia (ALL) cell lines
Panel of 14 human cancer cell lines; 72 h treatment at 0.01–10 µM; cell viability determined by luminescent detection of ATP</td><td>• broad sensitivity to selinexor-induced cytotoxicity, with no correlation between cell type (AML vs ALL) & sensitivity
<table>
<tr><th colspan="6">IC₅₀ values for inhibition of cell viability</th></tr>
<tr><th colspan="2">AML cell lines:</th><th colspan="2">T-ALL cell lines:</th><th colspan="2">B-ALL cell lines:</th></tr>
<tr><td>MOLM-13</td><td>21 nM</td><td>HPB-ALL</td><td>55 nM</td><td>HAL-01</td><td>115 nM</td></tr>
<tr><td>OCI-AML2</td><td>41 nM</td><td>DND-41</td><td>203 nM</td><td>UOCB-1</td><td>85 nM</td></tr>
<tr><td>MV4-11</td><td>46 nM</td><td>Jurkat</td><td>40 nM</td><td colspan="2" rowspan="5"></td></tr>
<tr><td>SKNO-1</td><td>63 nM</td><td>MOLT-4</td><td>34 nM</td></tr>
<tr><td>SKM-1</td><td>88 nM</td><td>SKW-3</td><td>123 nM</td></tr>
<tr><td>OCI-AML3</td><td>47 nM</td><td>KOPTK-1</td><td>71 nM</td></tr>
</table></td></tr>
<tr><td>Study KS-11 (KS-0011)
Cytotoxicity to human cancer cell lines
Panel of 45 human cancer cell lines; 72 h treatment at ≤3 µM; cell viability determined by MTS assay</td><td>
<table>
<tr><th colspan="2">Cell line</th><th>ED₅₀ (µM)</th><th>Killing at 3 µM (%)</th></tr>
<tr><td>bone:</td><td>HT-1080, U2OS</td><td>0.09, 1.11</td><td>82, 59</td></tr>
<tr><td>brain:</td><td>HTB-14, LN-229, T-98G</td><td>0.04, 0.08, 0.06</td><td>76, 72, 81</td></tr>
<tr><td>neuro-blastoma:</td><td>SY5Y, IMR-32, CHP-212, BE2C</td><td>0.06, 0.03, 0.07, 0.07</td><td>79, 100, 84, 100</td></tr>
<tr><td>breast:</td><td>AU-565, CAMA-1, ZR-75-1, HCC-1569, HCC-1937, BT-20, HS-578T, ZR-75-30, HCC-1500, HCC-2218</td><td>0.37, 0.22, 0.10, 0.09, 0.10, 0.83, 0.09, 0.04, 0.14, 0.03</td><td>98, 77, 52, 95, 63, 100, 69, 67, 64, 77</td></tr>
<tr><td>colon:</td><td>HXT-116 +/+, HCT-116 -/-, SW-480, SW-620, LoVo, Colo-201</td><td>0.06, 0.05, 0.05, 0.05, 0.21, 0.07</td><td>100, 97, 90, 85, 100, 82</td></tr>
<tr><td>liver:</td><td>HEP-3B, NCI-H2122, NCI-H1299, NCI-H889, NCI-H520, NCI-H226, A549</td><td>0.07, 0.04, 0.09, 0.03, 0.16, 0.07, 0.41</td><td>81, 100, 100, 57, 75, 80, 62</td></tr>
<tr><td>ovary:</td><td>CaOV3, OVCAR-5, OVCAR-8, OVCA-429</td><td>0.24, 0.09, 0.04, 0.14</td><td>71, 73, 77, 90</td></tr>
<tr><td>pancreas:</td><td>PANC-1, PACA-2</td><td>0.13, 0.10</td><td>38, 94</td></tr>
<tr><td>prostate:</td><td>PC-3, LnCAP, DU-145</td><td>0.17, <0.004, 0.33</td><td>98, 94, 80</td></tr>
<tr><td>skin:</td><td>A-375</td><td>0.08</td><td>96%</td></tr>
<tr><td>uterus:</td><td>MES-SA/DX5</td><td>0.06</td><td>81</td></tr>
<tr><td>renal sarcoma:</td><td>FUUR-1</td><td>0.49</td><td>100</td></tr>
<tr><td>alveolar soft sarcoma:</td><td>ASPS-KY</td><td>0.58</td><td>47</td></tr>
</table></td></tr>
</table>

cont.

Table 2.1. Summary of *in vitro* studies (cont.)

Study & experimental details	Major findings
Study KS-12 (KS-0012) *Cytotoxicity to mantle cell lymphoma (MCL) cell lines* Panel of 7 human MCL cell lines; 72 h treatment at 0.1 nM to 10 μM; cell viability determined by MTS assay	• all cell lines tested were sensitive to selinexor; median IC_{50} values: Jeko-1 48 nM Granta519 62 nM Mino 217 nM Z-138 358 nM SP53 21 nM NECB 567 nM Rec-1 3.4 nM
Study KS-13 (KS-0013) *Cytotoxicity & induction of mRNA expression in human leukocytes* Human leukocytes; 4 or 24 h treatment at 0.03–300 nM; cell viability determined by luminescent detection of ATP; quantitative real-time PCR of XPO1, MIC1, p53 & p21 mRNA	• selinexor (≤300 nM) had no effect of the viability of human leukocytes • selinexor induced XPO1 mRNA expression: — 2.1-, 3.8- & 4.9-fold at 3, 30 & 300 nM for 4 h — 2.2-, 2.9- & 3.0-fold at 3, 30 & 300 nM for 24 h • induction of MIC1 (≤22-fold), p53 (≤2.1-fold) & p21 (≤7.9-fold) mRNA with selinexor treatment also seen
Study KS-50014 *Activity of selinexor metabolite (KPT-375)* U2OS cells stably expressing Rev-GFP; 4 h treatment with selinexor & its trans-isomer, KPT-375, at 0.51 nM to 30 μM; nuclear/cytoplasmic localisation determined by fluorescence microscopy MM.1S cells treated with selinexor & KPT-375 (0–500 nM) for 1–1.5 h followed by determination of XPO1 occupancy using Simple Western	• KPT-375 showed 9.5-fold lower potency for inhibition of XPO1-mediated nuclear export of Rev-GFP *cf*. its parent — IC_{50} values, 88 nM for selinexor & 836 nM for KPT-375 • similarly, KPT-375 showed ~10-fold less efficient binding to XPO1 *cf*. selinexor in the occupancy assay — 50% occupancy at ~0.08 μM for selinexor & 0.7 μM for KPT-375
Study KS-50033 *Activity of four GSH-related selinexor metabolites* U2OS cells stably expressing Rev-GFP; 4 h treatment with KPT-5000, KPT-5003, KPT-5004 & KPT-5006 at 4.6 nM to 90 mM; nuclear/cytoplasmic localisation determined by fluorescence microscopy	• the four GSH-related metabolites of selinexor showed negligible pharmacological activity • IC_{50} values (*cf*. ~20 nM for selinexor in Study KS-01): KPT-5000 34.0 μM KPT-5003 35.3 μM KPT-5004 37.0 μM KPT-5006 36.3 μM
Turner *et al.* (2016) *Activity in combination with bortezomib* Proteasome inhibitor (PI)-resistant multiple myeloma cells; 20 h treatment with selinexor (300 nM), bortezomib (10 nM) or the two drugs in combination; induction of apoptosis assessed by activated caspase 3 expression	• synergistic induction of apoptosis in PI-resistant MM cells with selinexor & bortezomib in combination • immunofluorescence microscopy & Western blotting indicated inactivation of the NF-κB pathway by IκBα as the mechanism for sensitisation
Kashyap *et al.* (2016) *Mechanism for resistance & activity in combination with bortezomib* NF-κB expression in SINE-resistant HT-1080-R [fibrosarcoma] & ASPS-KY [alveolar soft part sarcoma] cells Cytotoxicity to U2OS cells treated with siRNA to silence IκBα, and to SINE-resistant HT-1080-R & ASPS-KY cells	• resistance to SINE compounds was correlated with ↑ basal NF-κB activity • silencing of IκBα (the cellular NF-κB inhibitor) resulted in a 65-fold ↓ in the cytotoxic potency of selinexor • treatment of SINE-resistant cells with 1 μM selinexor or 50 nM bortezomib alone induced low levels of apoptosis (HT-1080-R by bortezomib & ASPS-KY by both single agents), whereas the combination induced marked apoptosis

cont.

Table 2.1. Summary of *in vitro* studies (cont.)

Study & experimental details	Major findings
Argueta *et al.* (2018) *Effect on glucocorticoid receptor (GR) expression & activity in combination with dexamethasone* Expression of GR mRNA & protein in MM.1S cells Cytotoxicity to MM.1S, H929 (GR^+) & MM.1R (GR^{null}) cells (72 h treatment)	• selinexor induced expression of the glucocorticoid receptor (GR), and when combined with dexamethasone, increased GR transcriptional activity • cytotoxicity of selinexor to multiple myeloma cells appeared to be GR-independent (IC_{50} values of 40 & 50 nM in MM.1S & MM.1R cells) • synergistic cytotoxicity was seen with selinexor in combination with dexamethasone, dependent on GR expression

2.2. *IN VIVO* STUDIES

In vivo studies with selinexor as a single agent and in combination are summarised in Tables 2.2 and 2.3, respectively. Where other drugs were additionally tested in the studies, only data for established (*cf.* experimental) agents are reported for comparison.

Table 2.2. Summary of *in vivo* studies — monotherapy

Study & experimental details	Major findings
Study KS-13 (KS-0013) *Induction of XPO1 mRNA in rat leukocytes* **Rat** (SD); *n* = 3/sex; 2, 10 mg/kg given PO on days 1, 3 & 5 of each week for 2 weeks [total of 6 doses]; quantitative real-time PCR of leukocytes isolated from blood taken 24 h after the final dose	• mean ↑ in XPO1 mRNA: — 5.3- & 4.0-fold in ♂ & ♀ at 2 mg/kg — 7.1- & 5.0-fold in ♂ & ♀ at 10 mg/kg
Study KS-50001 *Induction of XPO1 mRNA in human leukocytes* Quantitative real-time PCR of XPO1 mRNA in leukocytes isolated from peripheral blood samples from **patients** with advanced, relapsed & refractory hematologic cancer receiving escalating doses of selinexor (3–57 mg/m² PO) in phase 1 Study KCP-330-001	• ↑ XPO1 mRNA in peripheral blood cells from haematological cancer patients, with peak effect (~4-fold induction) observed at ~4 h post-dose and levels staying elevated for up to 48 h • maximal induction obtained at ≥12 mg/m² (~20 mg) • the magnitude of XOP1 mRNA induction was not dependent on the tumour type (AML, MM, NHL/DLBCL)
Study KS-38 (KS-0038) *Anti-tumour efficacy [multiple myeloma]* **Mouse** (athymic nude), bearing MM.1S xenografts (initial tumour volume, ~350 or 1365 mm³); *n* = 5–8 ♀ Treatment groups (PO): • vehicle control • 10 mg/kg three times per week for the first week then 25 mg/kg twice weekly to day 60 • 15 mg/kg three times per week for 29 days • 15 mg/kg three times per week for the first two weeks then 25 mg/kg twice weekly to day 29	• significant attenuation of tumour development *cf.* vehicle control with treatment at 10/25 mg/kg for 60 days and at 15 mg/kg for 29 days (final tumour volumes, ~600 & 500 mm³ in the treated groups *cf.* 1900 & 1400 mm³ for vehicle) • treatment at 15/25 mg/kg reduced tumour size of more advanced tumours (declining from an initial tumour volume of 1365 mm³ to ~1000 mm³ by day 29)

cont.

Table 2.2. Summary of *in vivo* studies — monotherapy (cont.)

Study & experimental details	Major findings
Study KS-15 (KS-0015) *Anti-tumour efficacy [mantle cell lymphoma]* **Mouse** (SCID), bearing Z-138 MCL xenografts (initial tumour volume, ~200 mm^3); *n* = 5–7 ♀ Treatment groups (PO) [dose increases from day 10]: • vehicle control • 15 mg/kg three times weekly • 5/25 mg/kg three times weekly • 25 mg/kg three times weekly, commencing on day 10 • 25/40 mg/kg twice weekly • 35/40 mg/kg three times weekly • 25/40 mg/kg once weekly	• significant ↓ in tumour growth with treatment at 15 mg/kg three times weekly and at ≥25 mg/kg twice or three times weekly — *e.g.*, a decrease in tumour volume from 214 mm^3 on day 1 to 81 mm^3 on day 22 & to 75 mm^3 on day 30 with treatment at 15 mg/kg three times weekly *cf*. an increase from 196 mm^3 on day 1 to 2206 mm^3 on day 22 for vehicle controls
Study KS-18 (KS-0018) *Anti-tumour efficacy [chronic lymphocytic leukaemia]* **Mouse** (SCID), bearing Eμ-TCL1 murine leukemografts; *n* = 8–10 [sex not stated] Treatment groups (PO): • vehicle control • 5 mg/kg twice weekly • 15 mg/kg twice weekly • 3 mg/kg three times weekly • 10 mg/kg three times weekly • 1/15 mg/kg three times weekly [dose increase from week 9]	• significant ↑ in survival with treatment at 5 & 15 mg/kg twice weekly and at 10 & 15 mg/kg three times weekly, with effects at doses ≥10 mg/kg especially marked • no improvement in survival with treatment at 3 mg/kg three times weekly • body weight loss at ≥10 mg/kg three times weekly
Study KS-0112 *Anti-tumour efficacy [lymphoma]* **Mouse** (SCID), bearing DOHH-2 xenografts (initial tumour volume, 108–144 mm^3); *n* = 8 ♀ Treatment groups (PO): • vehicle • 7 mg/kg three times weekly • 10 mg/kg twice weekly • 10 mg/kg three times weekly	• significant attenuation of tumour growth in all treatment groups • 20%, 30% & 46% tumour growth delay [to day 25] and 41%, 30% & 51% tumour growth [at day 11] inhibition in the respective treatment groups
Study KS-0073 *Anti-tumour efficacy [T-cell acute lymphoblastic leukaemia]* **Mouse** (SCID), bearing MOLT-4 xenografts (initial tumour volume, 104–105 mm^3); *n* = 8 ♀ Treatment groups: • vehicle • 15 mg/kg PO three times weekly • 5 mg/kg doxorubicin IP once every 2 weeks	• ↓ tumour volume with treatment — 617 mm^3 with selinexor *cf*. 215 mm^3 with doxorubicin & 1716 mm^3 with vehicle on day 20 • body weight loss with treatment — loss of 6.4% for selinexor & 18.2% for doxorubicin to day 20 *cf*. a gain of 12.7% for vehicle controls
Study KS-0086 *Anti-tumour efficacy [acute myeloid leukaemia]* **Mouse** (NOD-SCID), bearing MV4-11 xenografts (initial tumour volume, ~120 mm^3); *n* = 8 ♀ Treatment groups: • vehicle • 15 mg/kg PO three times weekly	• ↓ tumour volume with treatment — 261 mm^3 with selinexor *cf*. 2052 mm^3 with vehicle on day 17 • ↓ body weight gain with treatment — 4.0% gain to day 17 with selinexor *cf*. 12.7% for vehicle control
Study KS-40 (KS-0040) *Anti-tumour efficacy [acute myeloid leukaemia]* **Mouse** (NSG; NOD-SCID-IL2Rcγ^{null}), bearing luciferase-expressing MV4-11 leukemografts; *n* = 9 ♀ Treatment groups: • vehicle • 15 mg/kg PO three times weekly • 25 mg/kg PO three times weekly	• marked suppression of leukaemic cell growth with treatment for 5 weeks at both dose levels, accompanied by ↑ animal survival (median survival, up to doubled) and with minimal toxicity to normal circulating blood cells & bone marrow cells evident • treatment produced weight loss, which was remediated by caloric supplementation

cont.

Table 2.2. Summary of *in vivo* studies — monotherapy (cont.)

Study & experimental details	Major findings
Study KS-87 (KS-0087) *Anti-tumour efficacy [acute myeloid leukaemia]* **Mouse** (NOD-SCID), bearing MOLM-16 xenografts (initial tumour volume, 83–84 mm^3); *n* = 8 ♀ Treatment groups: • vehicle • 15 mg/kg PO three times weekly	• ↓ tumour volume with treatment — 107 mm^3 with selinexor *cf*. 2676 mm^3 with vehicle on day 22 • body weight loss with treatment — loss of 0.2% for selinexor to day 22 *cf*. a gain of 28% for vehicle controls
Study KS-63 (KS-0063) *Anti-tumour efficacy [neuroblastoma]* **Mouse** (nude [nu/nu]), bearing IMR-32 or SHSY5Y xenografts (initial tumour volume, 94–104 & 100–112 mm^3); *n* = 10 ♂ Treatment groups: • vehicle • 10 mg/kg PO three times weekly • 20 mg/kg PO twice weekly • 20 mg/kg PO three times weekly • 5 mg/kg doxorubicin (IMR-32) or cisplatin (SHSY5Y) IP Q3W	• ↓ tumour volume with treatment — 399, 409 & 224 mm^3 in the respective selinexor groups *cf*. 556 mm^3 with doxorubicin & 1344 mm^3 with vehicle on day 29 for IMR-32 — 559, 708 & 453 mm^3 in the respective selinexor groups *cf*. 571 mm^3 with cisplatin & >1567 mm^3 with vehicle on day 29 for SHSY5Y • transient body weight loss with treatment — by 5.3–11.1% with selinexor *cf*. 5.1% & 5.8% with doxorubicin & cisplatin • ↓ overall body weight gain with treatment — gains of 6.9/6.4, 7.6/4.4 & 1.5/1.3% to the end of the study in the respective selinexor groups *cf*. 2.4/6.1% for doxorubicin/cisplatin & 7.3/8.5% for vehicle controls
Study KS-71 (KS-0071) *Anti-tumour efficacy [hepatocellular carcinoma]* **Mouse** (SCID), bearing Hep3B xenografts (initial tumour volume, 100–104 mm^3); *n* = 8 ♀ Treatment groups: • vehicle • 15 mg/kg PO three times weekly • 5 mg/kg doxorubicin IP once every 2 weeks	• ↓ tumour volume with treatment — 350 mm^3 with selinexor *cf*. 506 mm^3 with doxorubicin & 1559 mm^3 with vehicle on day 19 • no treatment-related effect on body weight — 11.9% loss of body weight to day 19 for vehicle controls *cf*. 9.4% loss for selinexor & 6.5% loss for doxorubicin
Study KS-72 (KS-0072) *Anti-tumour efficacy [colorectal carcinoma]* **Mouse** (SCID), bearing COLO 205 xenografts (initial tumour volume, 87–88 mm^3); *n* = 8 ♀ Treatment groups: • vehicle • 15 mg/kg PO three times weekly • 25 mg/kg 5-FU IP on days 1 & 3	• ↓ tumour volume with treatment — 250 mm^3 with selinexor *cf*. 447 mm^3 with 5-FU & 1217 mm^3 with vehicle on day 32 • no treatment-related effect on body weight with selinexor — 17.2% loss of body weight to day 32 for vehicle controls *cf*. 21.3% loss for selinexor & 5.8% loss for 5-FU
Study KS-67 (KS-0067) *Anti-tumour efficacy [non-small cell lung cancer]* **Mouse** (SCID), bearing A549 xenografts (initial tumour volume, 95–104 mm^3); *n* = 10 ♀ Treatment groups: • vehicle • 10 mg/kg PO three times weekly • 20 mg/kg PO three times weekly • 5 mg/kg cisplatin IP once every 2 weeks	• ↓ tumour volume with treatment — 630 & 359 mm^3 with 10 & 20 mg/kg selinexor *cf*. 1136 mm^3 with cisplatin & 1669 mm^3 with vehicle on day 29 • ↓ overall body weight gain with treatment — gains of 9.4% & 4.6% to day 29 in the selinexor groups *cf*. gains of 1.9% for cisplatin & 16.2% for vehicle controls

cont.

Table 2.2. Summary of *in vivo* studies — monotherapy (cont.)

Study & experimental details	Major findings
Study KS-14 (KS-0014) *Anti-tumour efficacy [prostate cancer]* **Mouse** (nude), bearing 22RV1 xenografts (initial tumour volume, 73–93 mm^3); $n = 10$ ♂ Treatment groups: • vehicle • 5 mg/kg PO 5 days weekly • 10 mg/kg PO 5 days weekly • 1/15 mg/kg PO 5 days weekly [dose increase from week 3] • 30 mg/kg cisplatin IP once every 4 weeks • 7.5 mg/kg docetaxel IP once weekly	• ↓ tumour volume with treatment — 490, 354 & 481 mm^3 in the respective selinexor groups *cf.* 891 & 671 mm^3 with cisplatin & docetaxel and 1263 mm^3 with vehicle on day 28 • weight loss of <2% over the course of the study with selinexor at ≤10 mg/kg & 12% at 15 mg/kg (*cf.* 14.6% & 8% losses with cisplatin & docetaxel and 23–30% gain for vehicle controls)

Table 2.3. Summary of *in vivo* studies — combination therapy

Study & experimental details	Major findings
Study KS-70 (KS-0070) *Anti-tumour efficacy [multiple myeloma]* **Mouse** (NOD-SCID), bearing MM.1S xenografts (initial tumour volume, 142–154 mm^3); $n = 8$ ♀ Treatment groups: • vehicle • selinexor: 5 mg/kg PO three times weekly • bortezomib: 0.5 → 0.2 mg/kg IP four days/week [dose reduction from week 2] • lenalidomide: 50 mg/kg PO daily • dexamethasone: 1 mg/kg IP five days/week • selinexor + bortezomib: 5/0.5 → 0.2 mg/kg • selinexor + lenalidomide: 5/50 mg/kg • selinexor + dexamethasone: 5/1 mg/kg	• mean tumour volume on day 17: — vehicle: 2232 mm^3 — selinexor: 1052 mm^3 — bortezomib: 2773 mm^3 — lenalidomide: 1097 mm^3 — dexamethasone: 1353 mm^3 — selinexor + bortezomib: 1230 mm^3 — selinexor + lenalidomide: 166 mm^3 — selinexor + dexamethasone: 244 mm^3 • selinexor in combination with lenalidomide or dexamethasone had a synergistic anti-tumour effect • bortezomib did not show anti-tumour activity as a single agent & did not enhance the activity of selinexor • Δ in body weight to day 17 in the respective groups: — vehicle: ↑ 12.7%; — single agents: ↓ 1.2%, ↑ 12.8%, ↑ 3.0%, ↓ 1.1% — combinations: ↓ 0.2%, ↓ 9.3%, 9.4%
Study KS-0085 *Anti-tumour efficacy [multiple myeloma]* **Mouse** (NOD-SCID), bearing H929 xenografts (initial tumour volume, 103–120 mm^3); $n = 9$ ♀ Treatment groups: • vehicle • selinexor: 5 mg/kg PO three times weekly • dexamethasone: 1 mg/kg IP daily • selinexor + dexamethasone: 5/1 mg/kg	• mean tumour volume on day 29: — vehicle: 1467 mm^3 — selinexor: 466 mm^3 — dexamethasone: 1287 mm^3 — selinexor + dexamethasone: 233 mm^3 • enhanced anti-tumour activity with selinexor & dexamethasone in combination • Δ in body weight to day 29 in the respective groups: — ↑ 13.6%, ↑ 16.0%, ↑ 20.8% & ↓ 3.3%
Study KS-68 (KS-0068) *Anti-tumour efficacy [multiple myeloma]* **Mouse** (NOD-SCID), bearing H929 xenografts (initial tumour volume, 96–105 mm^3); $n = 8$ ♀ Treatment groups: • vehicle • selinexor: 5 mg/kg PO three times weekly • selinexor: 10 mg/kg PO three times weekly • carfilzomib: 1.5 mg/kg IV two days/week • carfilzomib: 3 mg/kg IV two days/week • selinexor + carfilzomib: 5/1.5 mg/kg • selinexor + carfilzomib: 5/3 mg/kg • selinexor + carfilzomib: 10/1.5 mg/kg • selinexor + carfilzomib: 10/3 mg/kg	• mean tumour volume on day 23: (or earlier peak where individual animals were euthanised due to excessive tumour volume [>1500 mm^3]) — vehicle: >1833 mm^3 — selinexor groups: >1578 & 866 mm^3 — carfilzomib groups: >1321 & >1261 mm^3 — selinexor + carfilzomib groups: >904, >952, 563 & 449 mm^3 • enhanced anti-tumour activity with selinexor & carfilzomib (proteasome inhibitor) in combination • Δ in body weight to day 23 in the respective groups: — vehicle: ↑ 7.9% — selinexor: ↑ 7.3% & ↑ 1.8% — carfilzomib: ↑ 4.8% & ↑ 5.7% — combination: ↑ 3.5%, ↑ 3.0%, ↓ 2.6% & ↓ 4.0%

cont.

Table 2.2. Summary of *in vivo* studies — combination therapy (cont.)

Study & experimental details	Major findings
Study KS-108 (KS-0108) *Anti-tumour efficacy [multiple myeloma]* **Mouse** (nude), bearing MM.1S xenografts (initial tumour volume, 100–108 mm^3); $n = 8$ ♀ Treatment groups: • vehicle • selinexor: 5 mg/kg PO three times weekly • selinexor: 15 mg/kg PO three times weekly • panobinostat: 5 mg/kg IP five days/week • selinexor + panobinostat: 5/5 mg/kg	• mean tumour volume on day 18: — vehicle: 1715 mm^3 — selinexor groups: 784 & 446 mm^3 — panobinostat: 872 mm^3 — selinexor + panobinostat: 204 mm^3 • additive anti-tumour activity with selinexor & panobinostat (histone deactylase inhibitor) in combination • Δ in body weight to day 18 in the respective groups: — vehicle: ↑ 11.5% — selinexor: ↑ 5.6% & ↑ 7.2% — panobinostat: ↑ 4.8% — combination: ↓ 0.3%
Turner *et al.* (2016) *Anti-tumour efficacy [proteasome inhibitor-resistant multiple myeloma]* **Mouse** (NOD-SCID), bearing bortezomib-resistant U266PSR MM cells; $n = 5$ ♀ Treatment groups: • vehicle • selinexor: 10 mg/kg PO twice weekly • bortezomib: 0.5 mg/kg IP twice weekly • selinexor + bortezomib: 10/0.5 mg/kg	• selinexor showed significant anti-tumour activity as a single agent (*cf.* little effect of bortezomib) • synergistic anti-tumour activity with selinexor & bortezomib in combination (with mean tumour volumes with combination treatment less than half that with selinexor as a single agent)
Study KS-105 (KS-0105) *Anti-tumour efficacy [diffuse large B-cell lymphoma]* **Mouse** (nude), bearing Toledo xenografts (initial tumour volume, 102–109 mm^3); $n = 8$ ♀ Treatment groups: • vehicle • selinexor: 5 mg/kg PO three times weekly • selinexor: 15 mg/kg PO three times weekly • venetoclax: 25 mg/kg PO daily • venetoclax: 50 mg/kg PO daily • selinexor + venetoclax: 5/25 mg/kg • selinexor + venetoclax: 5/50 mg/kg	• mean tumour volume on day 29: — vehicle: 2153 mm^3 — selinexor groups: 1681 & 820 mm^3 — venetoclax groups: 126 & 52 mm^3 — selinexor + venetoclax groups: 47 & 20 mm^3 • enhanced anti-tumour activity with selinexor & venetoclax (BCL-2 inhibitor) in combination • Δ in body weight to day 30 in the respective groups: — vehicle: ↑ 12.8% — selinexor: ↑ 9.6% & ↓ 2.4% — venetoclax: ↑ 8.1% & ↑ 6.5% — combination: ↑ 2.1% & ↑ 6.2%
Study KS-0110 (KPM-69-Toledo) *Anti-tumour efficacy [diffuse large B-cell lymphoma]* **Mouse** (nude), bearing Toledo xenografts (initial tumour volume, 796–809 mm^3); $n = 10$ ♀ Treatment groups: • vehicle • selinexor: 5 mg/kg PO three times weekly • venetoclax: 25 mg/kg PO daily • selinexor + venetoclax: 5/25 mg/kg	• mean tumour volume on day 22: — vehicle: 3148 mm^3 — selinexor: 3177 mm^3 — venetoclax: 2109 mm^3 — selinexor + venetoclax: 1211 mm^3 • synergistic anti-tumour activity with selinexor & venetoclax in combination • Δ in body weight to day 22 in the respective groups: — ↑ 10.2%, ↑ 14.5%, ↑ 9.2% & ↑ 10.8%

cont.

Table 2.2. Summary of *in vivo* studies — combination therapy (cont.)

Study & experimental details	Major findings
Study KS-114 (KS-0114) *Anti-tumour efficacy [diffuse large B-cell lymphoma]* **Mouse** (SCID), bearing DoHH-2 xenografts (initial tumour volume, 116–118 mm^3); *n* = 10 ♀ Treatment groups: • vehicle • selinexor: 1 → 10[a] mg/kg PO three times weekly • selinexor: 2 → 7[a] mg/kg PO three times weekly • selinexor: 5 mg/kg PO three times weekly • selinexor: 15 → 10[b] mg/kg PO three times weekly • bendamustine: 25 mg/kg IV on day 1 • venetoclax: 25 mg/kg PO daily for 21 days • lenalidomide: 50 mg/kg PO daily • gemcitabine: 120 mg/kg IP once weekly • etoposide: 10 mg/kg IP once daily for 3 days • selinexor + bendamustine: 5/25 mg/kg • selinexor + venetoclax: 5/25 mg/kg • selinexor + lenalidomide: 5/50 mg/kg • selinexor + gemcitabine: 5/120 mg/kg • selinexor + etoposide: 5/10 mg/kg	• tumour growth delay: — selinexor groups: 18%, 4%, 11% & -1% — bendamustine: 38% — venetoclax: 1% — lenalidomide: 15% — gemcitabine: 57% — etoposide: 15% — selinexor + bendamustine: 61% — selinexor + venetoclax: 23% — selinexor + lenalidomide: 33% — selinexor + gemcitabine: 34% — selinexor + etoposide: 30% • enhanced anti-tumour activity observed with selinexor in combination with bendamustine, venetoclax, lenalidomide & etoposide, but not in combination with gemcitabine • mortality in 50% of mice treated with the highest dose level of selinexor, and in 60% of mice treated with selinexor + gemcitabine in combination
Study KS-61 (KS-0061) *Anti-tumour efficacy [breast cancer]* **Mouse** (SCID), bearing MDA-MB-231 xenografts (initial tumour volume, 160–174 mm^3); *n* = 8 ♀ Treatment groups: • vehicle • selinexor: 5 mg/kg PO three times weekly • selinexor: 10 mg/kg PO three times weekly • paclitaxel: 2 mg/kg IP on days 1, 3 & 5 • paclitaxel: 6 mg/kg IP on days 1, 3 & 5 • selinexor + paclitaxel: 5/2 mg/kg • selinexor + paclitaxel: 5/6 mg/kg • selinexor + paclitaxel: 10/2 mg/kg • selinexor + paclitaxel: 10/6 mg/kg	• mean tumour volume on day 27: — vehicle: 1940 mm^3 — selinexor groups: 1792 & 619 mm^3 — paclitaxel groups: 1912 & 888 mm^3 — selinexor + paclitaxel groups: 539, 437, 408 & 340 mm^3 • enhanced anti-tumour activity with selinexor & paclitaxel in combination • Δ in body weight to day 27 in the respective groups: — vehicle: ↑ 14.7% — selinexor: ↑ 11.3% & ↑ 8.4% — paclitaxel: ↑ 11.4% & ↑ 8.4% — combination: ↑ 8.6%, ↑ 12.0%, ↑ 5.8% & ↑ 9.1%

[a] = dose increase from week 3; [b] = dose reduction from week 4

3. SECONDARY PHARMACODYNAMICS

Selinexor was screened for secondary activity in a suite of 111 receptor binding and enzymatic assays at a concentration of 10 μM **(Study KS-34 [KS-0034])** and at up to 20 μM in two further kinase assays **(Study KS-65 [KS-0065])**. Notable activity (*i.e.*, ≥50% inhibition) was limited to inhibition of monoamine oxidase B (MAO-B), with 50% inhibition of radioligand binding observed with selinexor at 10 μM. Follow-up functional experiments, though, showed just under 25% inhibition of recombinant human MAO-B by selinexor at 10 μM **(Study KS-66 [KS-0066])**.

4. SAFETY PHARMACOLOGY

4.1. CNS EFFECTS

Study details	Experimental details; parameters measured	Results
Study KS-51 (KS-0051) WIL Research Laboratories, Ashland, OH, USA 26 July 2016 GLP	**Rat** (SD); *n* = 6 ♂ 0, 2, 10, 50 mg/kg PO (gavage) Functional observational battery (Irwin test) & body temperature; pre-dose, and 0.5, 2, 4 & 24 h post-dose	• no treatment-related effects on neurobehaviour • ↓ body temperature at HD (by 0.9°C *cf*. vehicle; at 4 h only)

4.2. CARDIOVASCULAR EFFECTS

Study details	Experimental details; parameters measured	Results
Study KS-55 (KS-0055) ChanTest Corporation, Cleveland, OH, USA 26 July 2016 GLP	hERG K^+ channels expressed in HEK293 cells 0, 1, 3, 10 & 30 µM; *n* = 3 assays conducted at 33–35°C; vehicle: 0.3% DMSO Peak tail current amplitude	• concentration-dependent inhibition: ↓ by 3.9, 10.4, 31.4 & 60.1% at 1–30 µM; • IC_{50} = 20.6 µM • assay sensitivity demonstrated with terfenadine as positive control

4.3. RESPIRATORY EFFECTS

Study details	Experimental details; parameters measured	Results
Study KS-54 (KS-0054) WIL Research Laboratories, Ashland, OH, USA 16 May 2012 Non-GLP	**Rat** (SD); *n* = 8 ♂ 0, 2, 10, 50 mg/kg PO Respiratory rate, tidal volume & minute volume by whole body plethysmography; monitoring 1 h pre-dose to 5 h post-dose	• effects seen at ≥MD, through to 5 h post-dose • ↓ respiration rate at ≥MD (peak ↓ of 16–19% *cf*. vehicle control across analysed intervals) • ↓ tidal volume at HD (by up to 19%) • ↓ minute volume at ≥MD (by up to 30–33%)

5. PHARMACODYNAMIC DRUG INTERACTIONS

Studies with selinexor in combination with bortezomib, dexamethasone and other anti-cancer agents are reported under *Primary pharmacology* (Section 2).

6. PHARMACOKINETICS

6.1. METHODS OF ANALYSIS

Quantification of selinexor in plasma was by validated LC-MS/MS assay. The lower limit of quantification (LLoQ) was 1.0 ng/mL for rat and monkey. Co-efficients of variation were ≤15% (≤20% at the LLoQ) and r^2 was >0.99.

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6.2. ABSORPTION AND PLASMA KINETICS

6.2.1. Single-dose studies

The plasma kinetics of selinexor were examined after a single PO and/or IV dose in male mice, rats, dogs and cynomolgus monkeys (Tables 6.1). More efficient initial oral absorption was evident in monkeys fasted overnight *cf*. fed animals (higher C_{max} and reached sooner), with no consistent effect on overall exposure (AUC).

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6.2.2. Repeat-dose studies

Pharmacokinetic/toxicokinetic parameters for selinexor after repeat oral dosing to rats and cynomolgus monkeys are presented in Tables 6.2 and 6.3. Peak and overall exposure (C_{max} and AUC) were approximately dose-proportional. No sex difference was apparent, nor notable accumulation with repeat dosing.

The 13-week study in rats and the 4- and 13-week studies in monkeys included measurement of the trans-isomer of selinexor, KPT-375. Plasma C_{max} and $AUC_{0-24\,h}$ values for KPT-375 were ≤1.7% and ≤3.2% of that for selinexor (cis-isomer) in rats. Peak plasma levels of KPT-375 in the 4-week monkey study were 0.92–1.7% (mean, 1.2%) that of selinexor, and plasma C_{max} and $AUC_{0-24\,h}$ values for KPT-375 in the 13-week monkey study were 0.34–1.6% and 0.15–1.4% that for selinexor.

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6.2.3. Plasma kinetics in human subjects

Pharmacokinetic parameters for selinexor in cancer patients at clinically proposed doses are shown in Table 6.4. The apparent volume of distribution in humans at steady state was reported to be 133 L.

Table 6.4. Pharmacokinetic parameters in humans

Study & sampling details	Dose (PO)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-24\,h}$ (ng·h/mL)	$t_{½}$ (h)
Clinical Study KCP-330-001 (Phase 1) Patients with advanced haematological malignancies; 6 time points over 0.5–24 h post-dose on cycle 1 / day 1; *n* = 4–9	80 mg#	680	1.9	5386	5.8
	100 mg#	693	1.9	6998	6.0

= approximated from references to doses of 46 and 60 mg/m² in ~70 kg patients

6.3. DISTRIBUTION

6.3.1. Protein binding

Protein binding by selinexor was high in mouse, rat, monkey and human plasma, and moderate in dog plasma, and independent of concentration (Table 6.5).

Table 6.5. Plasma protein binding by selinexor

Experimental details	Species	Study KS-25 (KS-0025)	Study KS-50013			
		Binding at drug concentration				
		1 µM	0.01 µM	0.1 µM	1 µM	10 µM
Equilibrium dialysis; 4–5 h incubation at 37°C; *n* = 2–3	mouse	95.0%	–	–	–	–
	rat	96.3%	>78.1%	97.2%	96.8%	96.2%
	dog	54.2%	–	–	–	–
	monkey	96.2%	>86.1%	95.8%	95.6%	95.6%
	human	–	–	>95.4%	95.4%	95.1%

– = not determined

Blood:plasma ratios in rat, monkey and human blood were <1 (Table 6.6), indicating no preferential distribution of selinexor into red blood cells.

Table 6.6. Red blood cell partitioning by selinexor

Study	Experimental details	Species	Blood:plasma ratio at drug concentration			
			0.01 µM	0.1 µM	1 µM	10 µM
KS-50013	15 min incubation at 37°C; *n* = 3	rat	0.80	0.80	0.84	0.90
		monkey	0.81	0.79	0.79	0.80
		human	0.66	0.63	0.69	0.66

– = not determined

6.3.2. Tissue distribution

Tissue distribution of radioactivity in rats after PO administration of ^{14}C-selinexor was rapid and wide (Table 6.7). Penetration of the blood-brain barrier by selinexor was shown in mice, rats and monkeys.

Table 6.7. Tissue distribution of ^{14}C-selinexor-derived radioactivity

Study & experimental details	Findings
Study KS-91 (KS-0091; WIL-859039) **Rat** (SD) 4 mg/kg ^{14}C-selinexor PO (gavage) Liquid scintillation counting of tissue samples taken from animals euthanised at 1, 2, 4, 8, 24, 48 & 168 h post-dose; *n* = 1 ♂/time point	• tissue T_{max} values were typically 1–2 h • tissue:plasma C_{max} ratios were highest in the small intestine (7.4), kidney medulla (5.5), stomach (5.2), kidney (4.0) & liver (3.8) and lowest in the eye (0.03), bone (0.27), testis (0.32), brain (0.33), spinal cord (0.39), uveal tract (0.40) & skin (0.51) • levels of radioactivity in tissues generally declined in parallel with plasma, with substantial elimination observed at 168 h (average final concentration, ~4% of peak)
Studies KS-19 (KS-0019), KS-20 (KS-0020) & KS-21 (KS-0021) **Mouse** (CD-1), **Rat** (SD) & **Monkey** (Cynomolgus) 10 mg/kg selinexor PO (gavage) LC-MS/MS of plasma & brain samples from animals euthanised at 2 h post-dose; *n* = 2–3 ♂	• brain:plasma selinexor concentration ratios were 0.71 for mice, 0.72 for rats & 0.61 for monkeys

6.4. METABOLISM

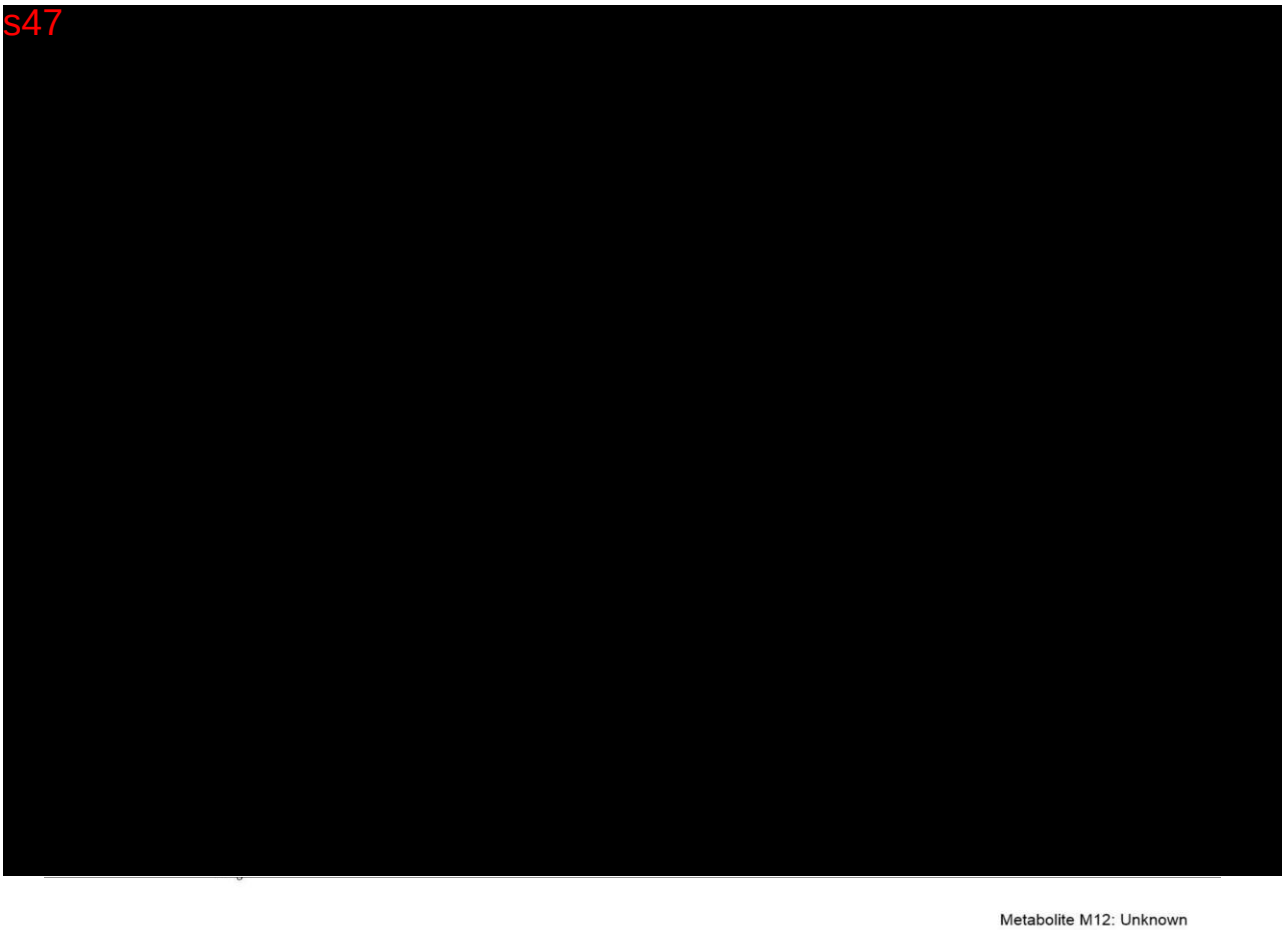
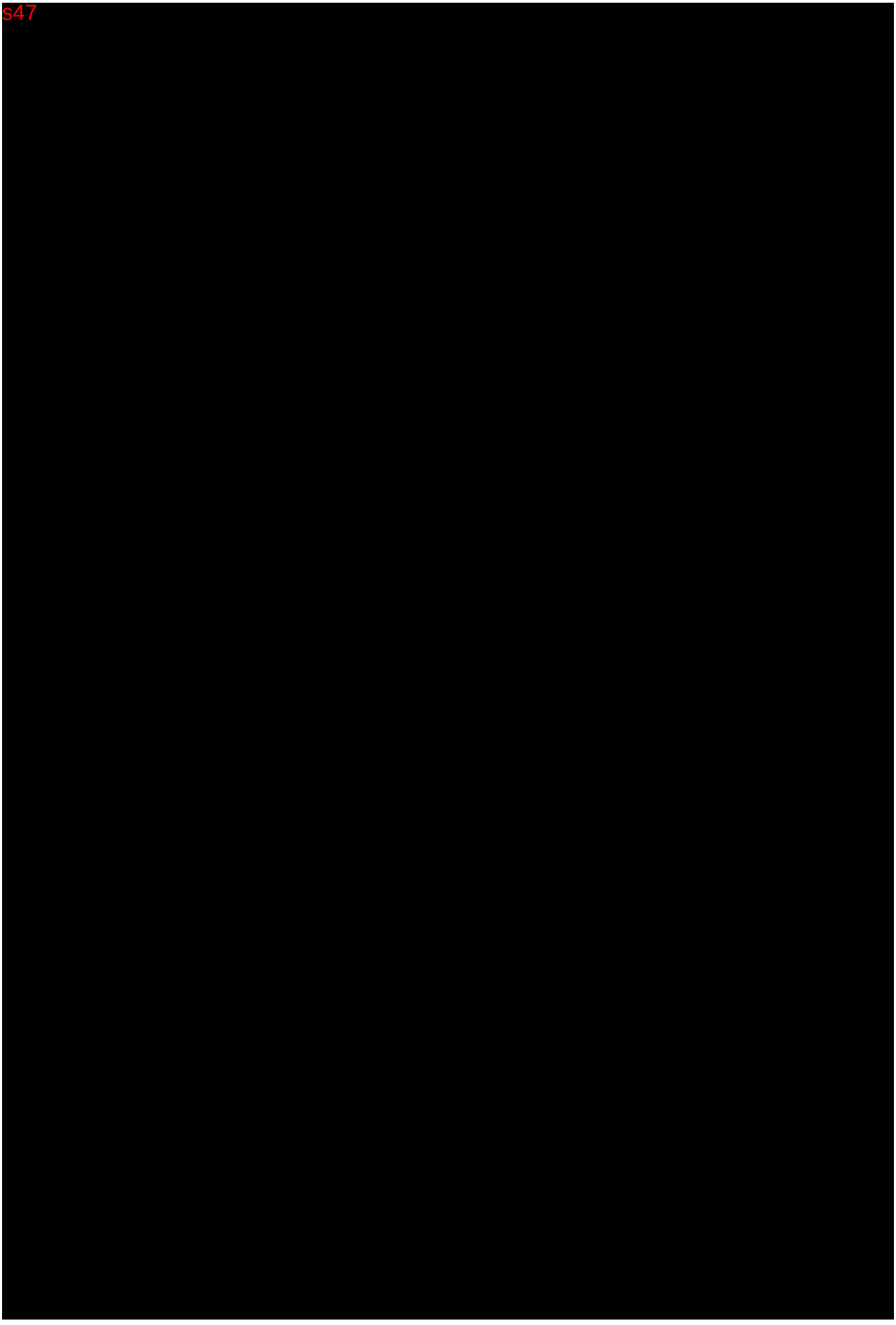


Figure 6.2. Proposed metabolic pathway for selinexor in rats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6.4.2. *In vitro* studies

6.4.2.1. Metabolism in microsomes and hepatocytes

Metabolic stability

Selinexor (1 μM) was incubated with mouse, rat, dog, monkey and human liver microsomes (0.5 mg protein/mL) in the presence of NADPH for up to 45 min at 37°C **(Study KS-26 [KS-0026])**. At the end of incubation period, 78.2–90.4% selinexor remained. Extrapolated half-lives were 3.50, 3.19, 2.08, 4.19 and 3.78 h for the respective species.

Selinexor (1 μM) was also incubated with human liver S9 fraction in the presence of various cofactors (NADPH, UDP-glucuronic acid, and 3-phospho adenosine 5-phospho sulfate) for up to 45 min at 37°C **(Study KS-27 [KS-0027])**. At the end of the incubation period, 89% selinexor remained. The extrapolated half-life was 6.2 h.

Metabolic profile

^{14}C-Selinexor (10 μM) was incubated with liver microsomes prepared from mice, rats, dogs, monkeys and humans (1 mg protein/mL) for 30 min **(Study KS-29 [KS-0029])** and with human hepatocytes (2×10^6 cells/mL) for 4 h **(Study KS-30 [KS-0030])** at 37°C followed by LC-UV and LC-MS to identify the metabolites formed. No metabolites of selinexor were detected in mouse, rat, dog, monkey or human liver microsomes. Two metabolites were observed in human hepatocyte incubations: a glucuronide (present at ~4.5% of the initial parent level) and a GSH conjugate (at ~2.5%).

Selinexor (10 μM) was incubated with human liver microsomes in the presence of NADPH and using GSH as a trapping agent, with LC-UV-MS analysis performed following 0 and 60 min incubation at 37°C **(Study K-31 [KS-0031])**. One GSH conjugate was detected at both time points and in similar amounts (indicating that its formation is not microsome-dependent).

6.4.2.2. Identification of selinexor-metabolising CYP and UGT isoforms

Selinexor (0.1 or 1 μM) was incubated with recombinant human CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 or 3A4 (100 pmol/mL) **(Study KS-50008)** or with recombinant human UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7 of 2B15 (0.25 mg protein/mL) **(Study KS-50009)** for 2 h at 37°C. Of the tested CYP isozymes, notable metabolism was only observed with CYP3A4 (28–32% loss of selinexor). With UGT isozymes, greatest loss of selinexor was observed with incubation with UGT1A1, 1A3 and 1A9 (21–23% loss) at 0.1 μM, and with UGT1A1 and 1A6 (20–22% loss) at 1 μM, although substrate loss above control levels was observed for most of the other UGT isozymes as well.

6.4.3. *In vivo* studies

Three circulating metabolites of selinexor were detected in **rats** (SD; *n* = 3 males) following PO administration of ^{14}C-selinexor **(Study KS-91 [KS-0091])** (Table 6.8). Exposure to unchanged drug dominated.

Table 6.8. Metabolites of selinexor in rat plasma

Metabolite	Relative percent in plasma following PO administration									
	0.25 h	0.5 h	1 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h
Selinexor [unchanged drug]	87.9	90.1	90.9	85.9	81.2	58.9	35.5	4.0	1.7	3.3
M1 [glucuronide]	4.5	4.0	1.8	1.5	4.3	0.4	0.2	0.6	1.0	2.0
M2 [cysteine conjugate]	7.6	5.9	7.2	12.6	8.7	1.9	1.4	3.0	3.3	2.0
M16 [$(CF_3)_2$-phenyl-COOH]	–	–	0.1	–	5.8	38.8	62.9	92.3	93.9	92.7

– = not present

Sixteen metabolites were identified in **rat** urine. Unchanged drug represented ≤8.8% of sample radioactivity at any time point and an average of 2.0% across the time points studied (6, 12, 24, 48, 72, 96 and 120 h). Faeces (12, 24 and 48 h) contained a single metabolite [M15; $(CF_3)_2$-phenyl-triazole], present at 79–96%, along with unchanged selinexor.

A cysteine conjugate was detected in the plasma of **cynomolgus monkeys** dosed at 2.5 mg/kg PO three times weekly (plasma collected pre-dose and 2, 6 and 24 h post-dose on days 1 and 26 in Study KS-46 [KS-0046]), with 5.4% relative abundance (*cf*. 94.6% for unchanged selinexor) **(Study KS-32 [KS-0032)**. Urine (0–24 h) contained three GSH-related metabolites — cysteine, N-acteylcysteine and glycine-cysteine conjugates (representing 88%, 8% and 1.4% of the sample, respectively) — as well as unchanged selinexor (representing 2.8% of the drug-related material).

Plasma from **cynomolgus monkeys** dosed at 3 mg/kg PO three times or twice weekly (pooled from samples collected pre-dose, 1, 2, 8, 12 and 24 h post-dose on day 40 or 60 in Study KNC-N-18-001) contained unchanged selinexor as the clearly predominant moiety **(Study KS-50015)**. Ten circulating metabolites were observed: M1 (cysteine conjugate), M2 (glutathione conjugate), M3 (cysteinylglycine conjugate), M4 (N-dealkylation + oxidation), M5 (N-glucuronidation), M6 (oxidation + glucuronidation), M7 (N-dealkylation), M8 (trans-isomer of selinexor), M9 (amide hydrolysis) and M10 (M7-related).

An exploratory metabolite scan study **(Study KS-50031)** was performed using plasma and urine obtained from **patients** with haematological (Study KCP-330-001) or solid tumour (Study KCP-330-002) malignancies following repeated oral administration of selinexor at dose levels ranging from 3 to 30 mg/m^2. In plasma, individual metabolites accounted for less than 1% of parent at peak selinexor plasma concentrations. Identified circulating metabolites comprised two glucuronides (of the parent drug and hydroxylated parent), hydroxylated selinexor, and cysteine and cysteine-glycine conjugates. Two glucuronides of selinexor were observed in urine, along with some unchanged drug.

From plasma samples obtained from **patients** across the clinical program, unchanged selinexor was identified as the major circulating moiety, with the trans-isomer of selinexor (M8 [KPT-375]) the most common circulating metabolite (<5% of peak of parent levels) [as reported in the Clinical Overview].

Analysis of urine and faeces samples collected from **patients** in Study KCP-330-003 revealed a total of six metabolites **(Study KS-50034)**. In human urine, the predominant metabolite identified was formed by cysteine conjugation (M1). Other minor pathways included direct N-glucuronidation (M7a), N-dealkylation (M7), N-dealkylation followed by glucuronidation (M2a), and N-dealkylation with oxidation followed by glucuronidation (M3a). The predominant metabolite identified in human faeces was formed by N-dealkylation (M7). Selinexor and its trans-isomer (M8 [KPT-375]) were detected in both urine and faeces.

6.5. EXCRETION

Studies in rats showed excretion predominantly via the faeces/bile (Table 6.9).

Table 6.9. Excretion into urine, faeces and bile

Study & experimental details	Collection period	Analyte	Percent dose			
			Urine	Faeces	Bile	Total[#]
Study KS-91 [KS-0091; WIL-859039] **Rat** (SD); *n* = 3 ♂ 5 mg/kg ^{14}C-selinexor PO	0–12 h	radioactivity	3.5	12.3	–	16.2
	0–24 h		7.6	44.3	–	52.8
	0–48 h		12.8	70.5	–	84.6
	0–120 h		15.9	74.1	–	91.7
	0–168 h		16.3	74.7	–	92.7
Study KS-53 [KS-0053] **Rat** (SD; bile-duct cannulated); *n* = 3 ♂ 10 mg/kg selinexor PO	0–24 h	selinexor	0.11	1.56	2.65	4.32
		cysteine conjugate	0.38	1.58	29.9	31.8
		GSH conjugate	0.01	0.005	15.2	15.2
		glycine-cysteine conjugate	1.03	0.016	17.6	18.7
		N-acetylcysteine conjugate	0.44	0.02	1.93	2.39
		parent + metabolites	1.97	3.18	67.3	72.4

[#] = includes cage rinse for Study KS-91

Unchanged selinexor recovered in urine of male cynomolgus monkeys to 24 h after dosing at 2.5 mg/kg accounted for 0.094% of the dose **(Study KS-46 [KS-0046])**. According to the Clinical Overview, based on quantitation of selinexor in human urine in Study 003, urinary excretion is a minor elimination pathway for selinexor.

6.6. PHARMACOKINETIC DRUG INTERACTIONS

6.6.1. Enzyme inhibition by selinexor

Selinexor was investigated for CYP and UGT inhibitory activity in experiments with human liver microsomes performed at 37°C (Tables 6.10 and 6.11). CYP inhibition was direct (*cf*. time- or metabolism-dependent), with CYPs 2B6 and 3A4/5 the most sensitive isozymes. UGT1A1 was the UGT isozyme most sensitive to inhibition by selinexor (but the IC_{50} exceeds 50 µM).

Table 6.10. CYP inhibition by selinexor in human liver microsomes

Study	CYP isozyme	Probe substrate	IC_{50} or maximum inhibition (≤50 µM)		
			Direct inhibition[a]	Time-dependent inhibition[b]	Metabolism-dependent inhibition[c]
KS-50010	1A2	phenacetin	13%	17%	19%
	2B6	efavirenz	24 µM	30 µM	17 µM
	2C8	amodiaquine	~50 µM	~50 µM	40 µM
	2C9	diclofenac	42 µM	~50 µM	43 µM
	2C19	*S*-mephenytoin	35 µM	39 µM	35 µM
	2D6	dextromethorphan	21%	15%	23%
	3A4/5	midazolam	10%	3%	19%
		testosterone	24 µM	43 µM	25 µM

[a] = no pre-incubation; [b] = 30 min pre-incubation without NADPH; [c] = 30 min pre-incubation with NADPH

Table 6.11. UGT inhibition by selinexor in human liver microsomes

Study	UGT isozyme	Probe substrate	Maximum inhibition (≤50 µM)
KS-50011	1A1	17β-estradiol	47%
	1A3	chenodeoxycholic acid	5.5%
	1A4	trifluoperazine	7.9%
	1A6	1-naphthol	none
	1A9	propofol	20%
	2B7	morphine	13%

6.6.2. Enzyme induction by selinexor

Three cultures of primary human hepatocytes were exposed to selinexor (0.3–10 µM; replenished daily), vehicle only or positive control agents (omeprazole, phenobarbital, rifampin) for three days followed by measurement of CYP1A2, CYP2B6 and CYP3A4 mRNA levels **(Study KS-50007)**. The highest tested concentration was the maximum feasible based on cytotoxicity. Induction was observed for CYP1A2 in one of three cultures (by 5.1-fold; equivalent to 3.5% of the positive control response). Selinexor did not induce CYP2B6 or CYP3A4.

6.6.3. Transporter interactions

Selinexor showed high permeability ($P_{app} > 10 \times 10^{-6}$ cm/s) in experiments with Caco-2 **(Study KS-0023)** and MDCK-MDR1 **(Studies KS-0119 and KS-50012)** cell monolayers.

Results of experiments performed in transfected cells investigating selinexor as a substrate and inhibitor of major transporters are summarised in Table 6.12 **(Study KS-50012)**. Selinexor was seen to be a weak substrate of BCRP at 0.1 µM, with an efflux ratio of 2.18, which was reduced in the presence of known BCRP inhibitors (Ko143 and lopinavir); the efflux ratio at 1 µM, though, was below the threshold (1.89 *cf.* 2) for the drug to be considered a substrate for the transporter.

Table 6.12. Transporter interactions by selinexor

Cell line	Transporter	Selinexor as substrate	Selinexor as inhibitor	
			Probe substrate	IC_{50} or maximum inhibition
MDCKII	P-glycoprotein	no	–	–
	BCRP	weak/no	–	–
HEK293	OATP1B1	no	^{3}H-estradiol-17β-glucuronide	11.2 µM
	OATP1B3	no		6.2 µM
	OAT1	no	^{3}H-p-aminohippurate	35.6 µM
	OAT3	no	^{3}H-estrone-3-sulfate	11.2 µM
	OCT1	no	–	–
	OCT2	no	^{14}C-metformin	8.9% at 30 µM
	MATE1	no		22.3 µM
	MATE2-K	no		none at 30 µM

– = not investigated

7. SINGLE-DOSE TOXICITY

The acute oral toxicity of selinexor was investigated in a study in rats (Table 7.1).

Table 7.1. Summary of single-dose toxicity study

<table>
<tr><th>Study & experimental details</th><th>Major findings</th></tr>
<tr><td>Study KS-41 (KS-0041)
Shanghai ChemPartner Co. Ltd, Shanghai, China
26 July 2016
Non-GLP

Rat (SD); n = 3/sex
0, 25, 100, 500 mg/kg PO (gavage); vehicle: 0.5% Pluronic F68
24 h observation</td><td>
Mortality: none
Clinical signs:
• diarrhoea at ≥MD [mild at MD; severe at HD]
• ↓ motor activity, piloerection & cold to touch at HD
Body weight:
• dose-dependent loss at all dose levels (by 7.4%, 8.2% & 10.9% in ♂ and by 3.9%, 3.8% & 5.1% in ♀; accompanied by ↓ food & water consumption)
Serum chemistry:
• ↑ LDH at all dose levels (2.6–3.0× control in ♂ & 3.1–4.3× control in ♀)
• ↑ creatine kinase at all dose levels (1.9–2.6× control in ♂ & 2.3–3.1× in ♀)
• ↑ AST in HD ♂ (3.6× control) and at ≥MD in ♀ (2.1–3.3× control)
• ↑ ALT at HD (2.0× control in ♂ & 1.9× control in ♀)
• ↑ BUN at HD (6.0× control in ♂ & 4.4× control in ♀)
• ↑ creatinine in HD ♂ (2.6× control)
• ↑ amylase in HD ♀ (2.2× control)
Gross pathology:
• thin stomach & intestine walls and stomach & intestines filled with liquid content at ≥MD; stomach enlargement at HD
Toxicokinetics:
• plasma sampling at 1, 3, 8 & 24 h post-dose (n = 3/sex/time point):
<table>
<tr><th rowspan="2">Dose (mg/kg)</th><th colspan="2">C_{max} (ng/mL)</th><th colspan="2">T_{max} (h)</th><th colspan="2">$AUC_{0-24\,h}$ (ng·h/mL)</th></tr>
<tr><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th></tr>
<tr><td>25</td><td>7480</td><td>4647</td><td>1.0</td><td>1.0</td><td>21633</td><td>12843</td></tr>
<tr><td>100</td><td>14633</td><td>17200</td><td>1.0</td><td>1.0</td><td>64400</td><td>67400</td></tr>
<tr><td>500</td><td>26900</td><td>28567</td><td>1.7</td><td>1.0</td><td>204667</td><td>185667</td></tr>
</table>
• brain:plasma concentration ratios at 24 h post-dose: 0.72, 0.58 & 1.15 for ♂ & 0.75, 0.68 & 0.48 for ♀
Maximum non-lethal dose: 500 mg/kg PO
</td></tr>
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8. REPEAT-DOSE TOXICITY

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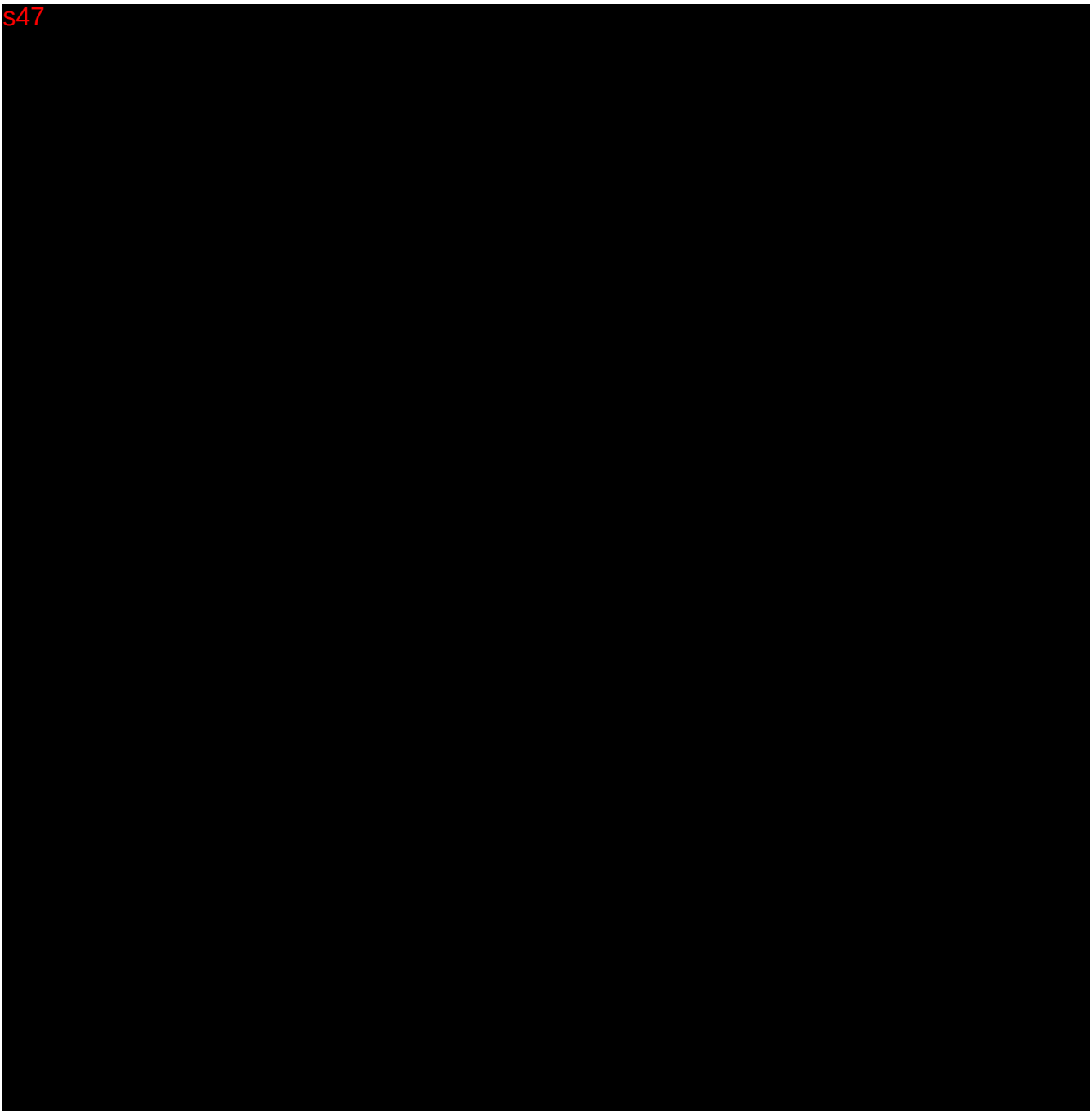
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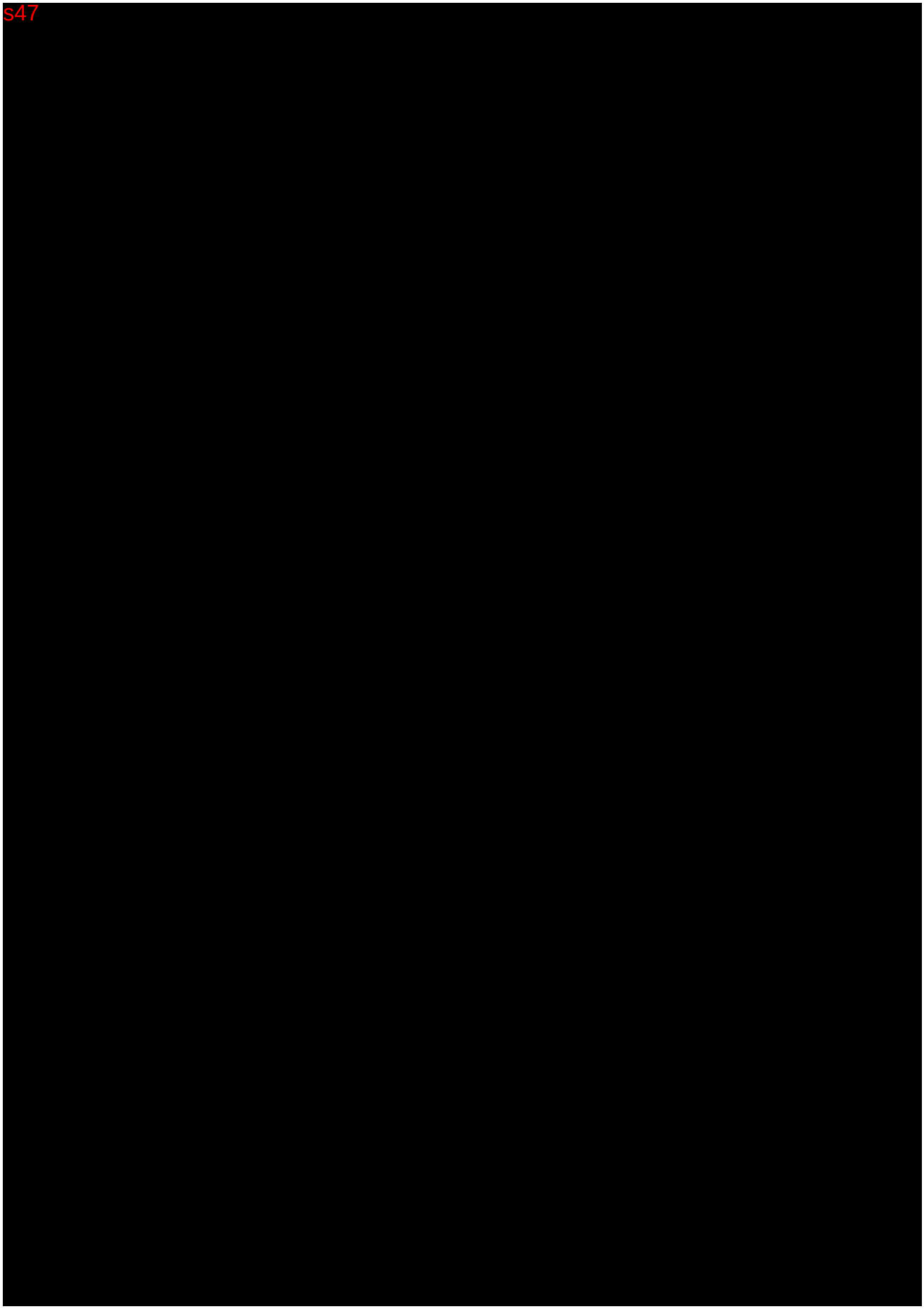

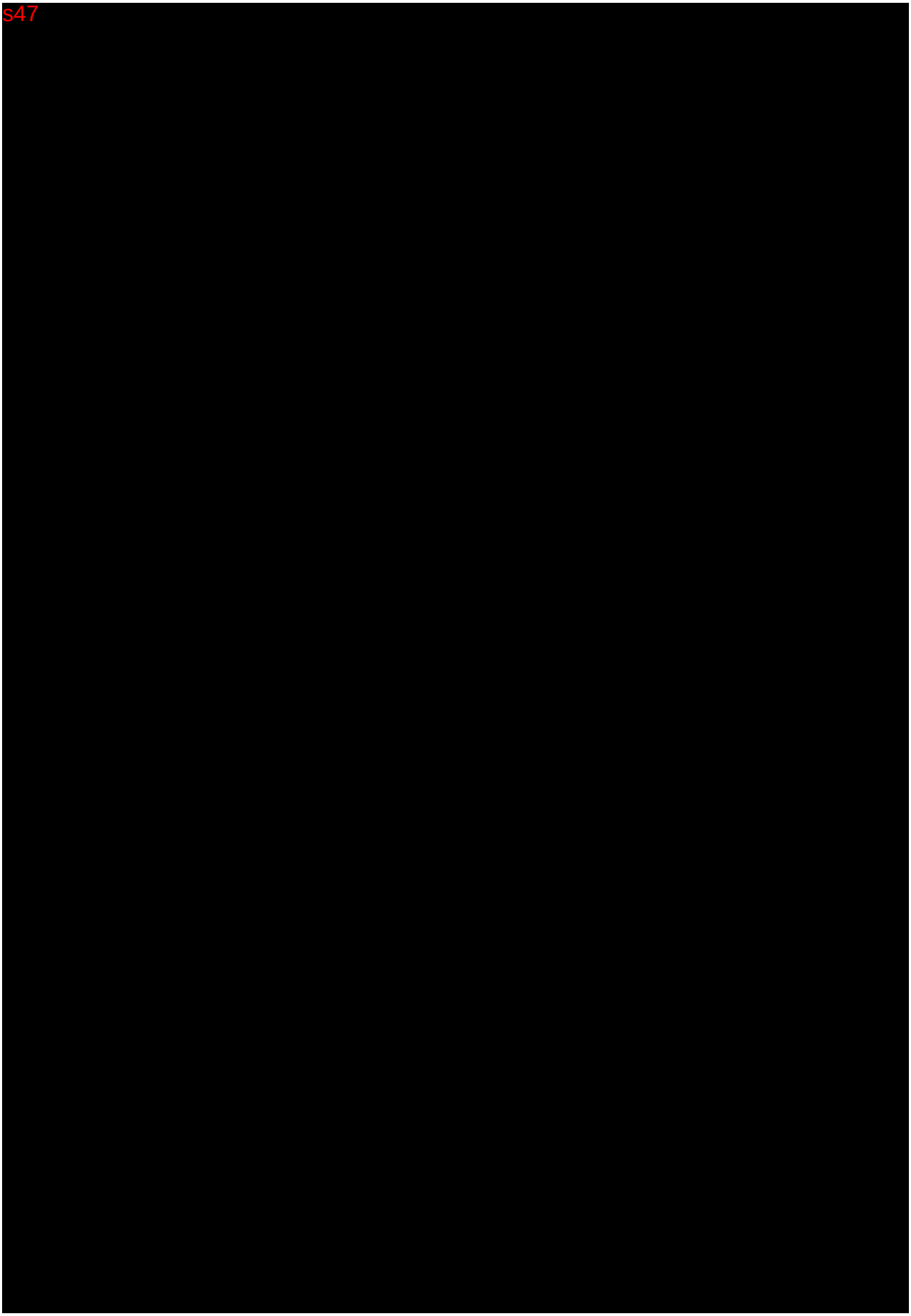
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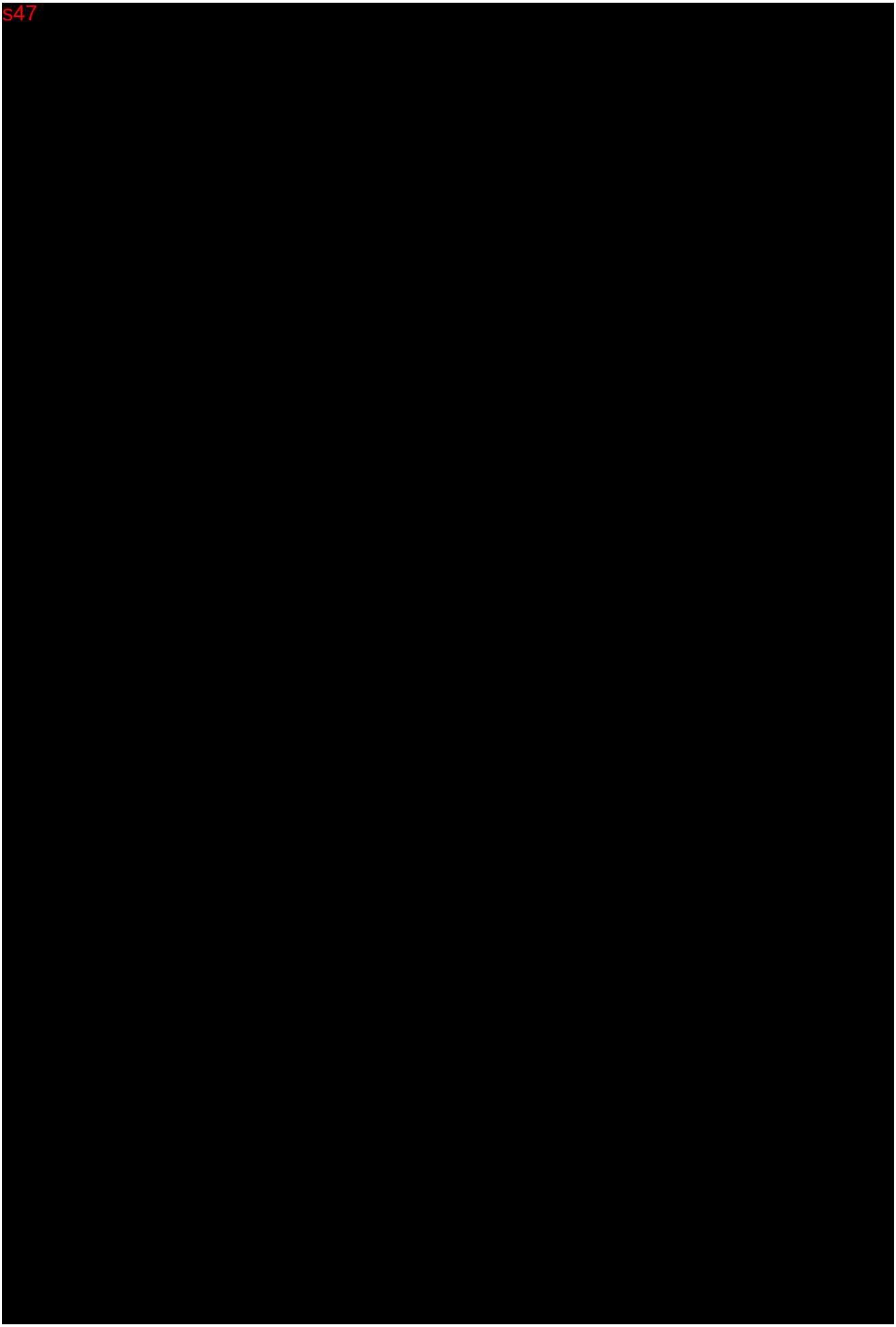

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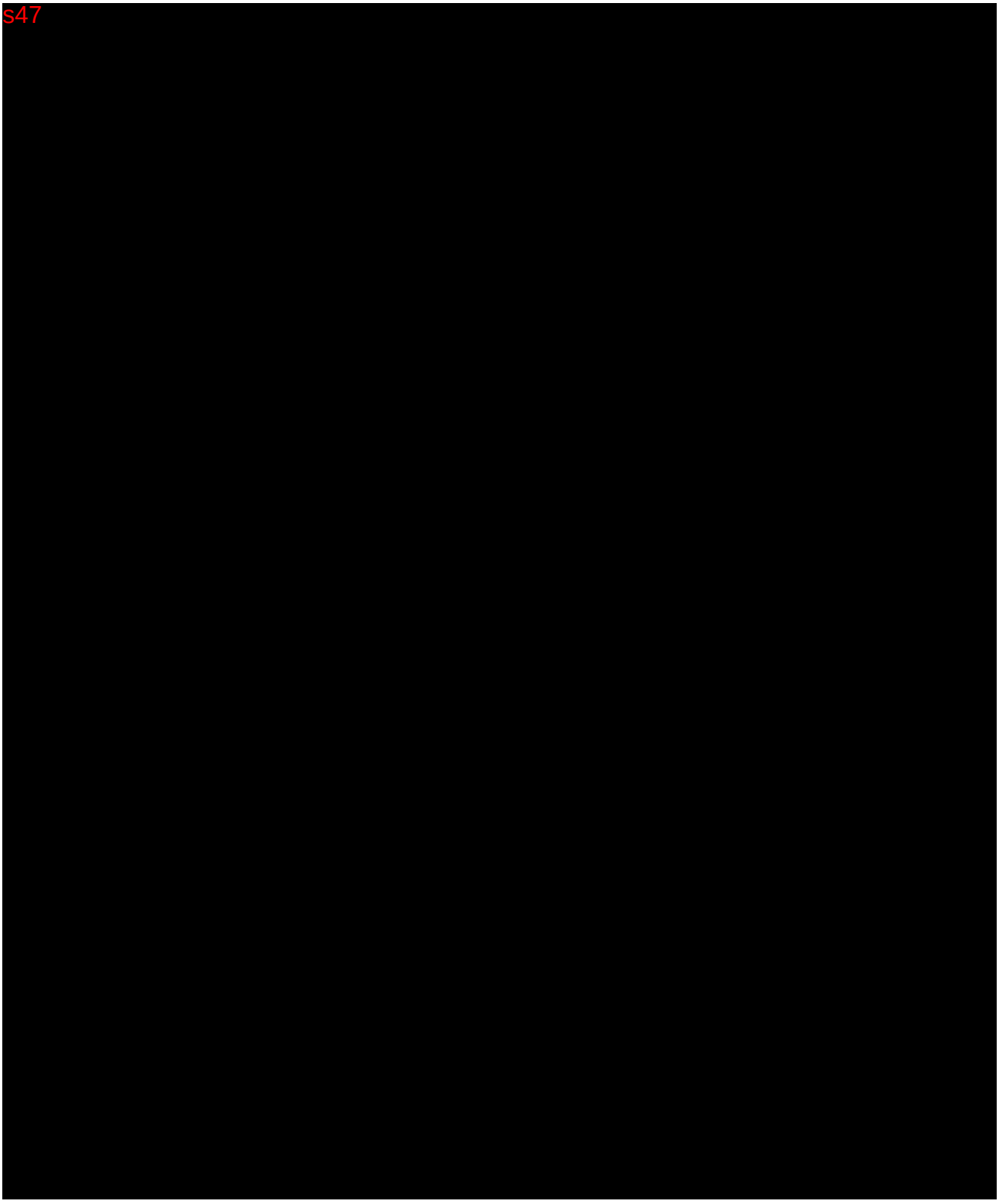

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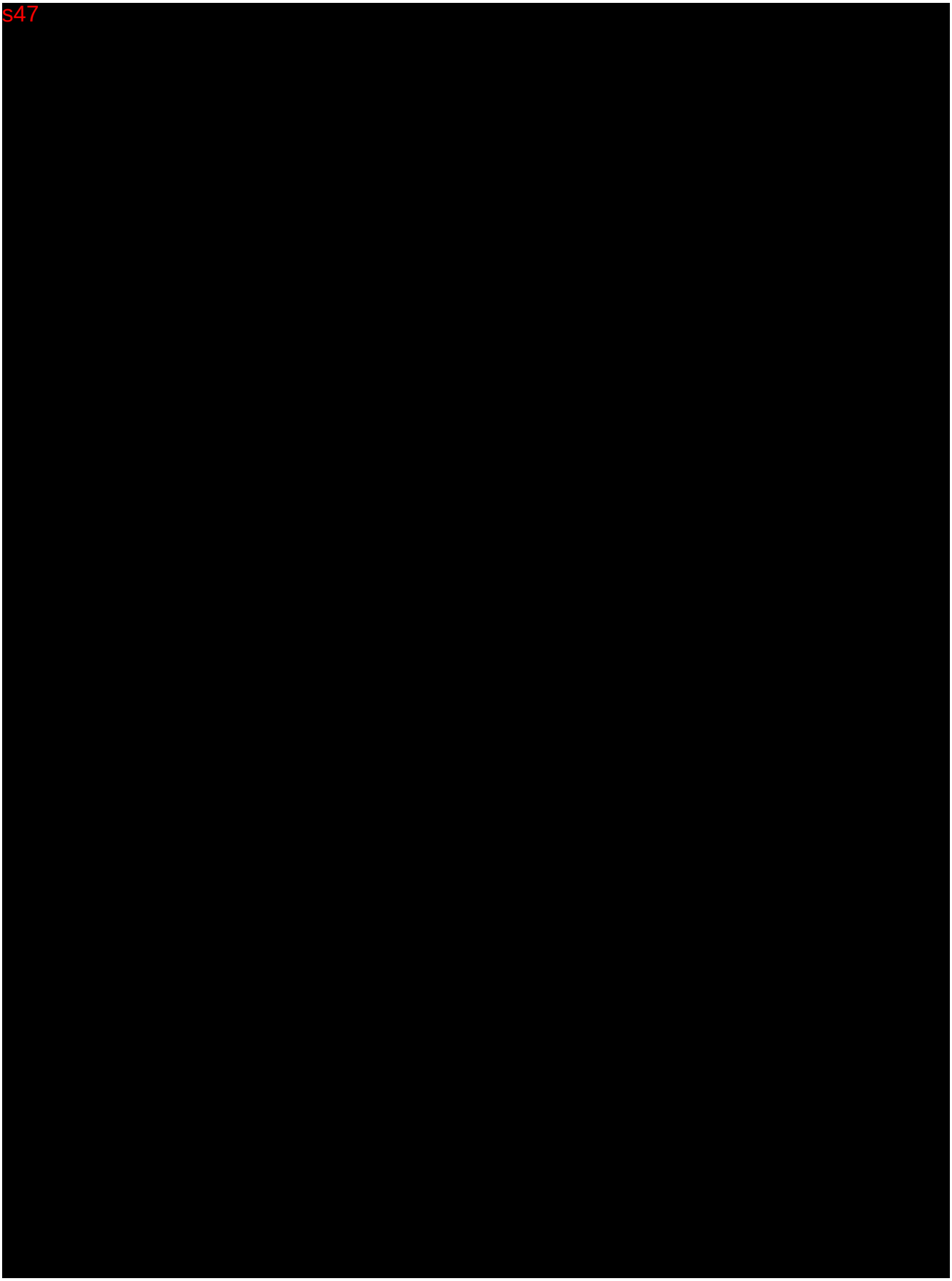

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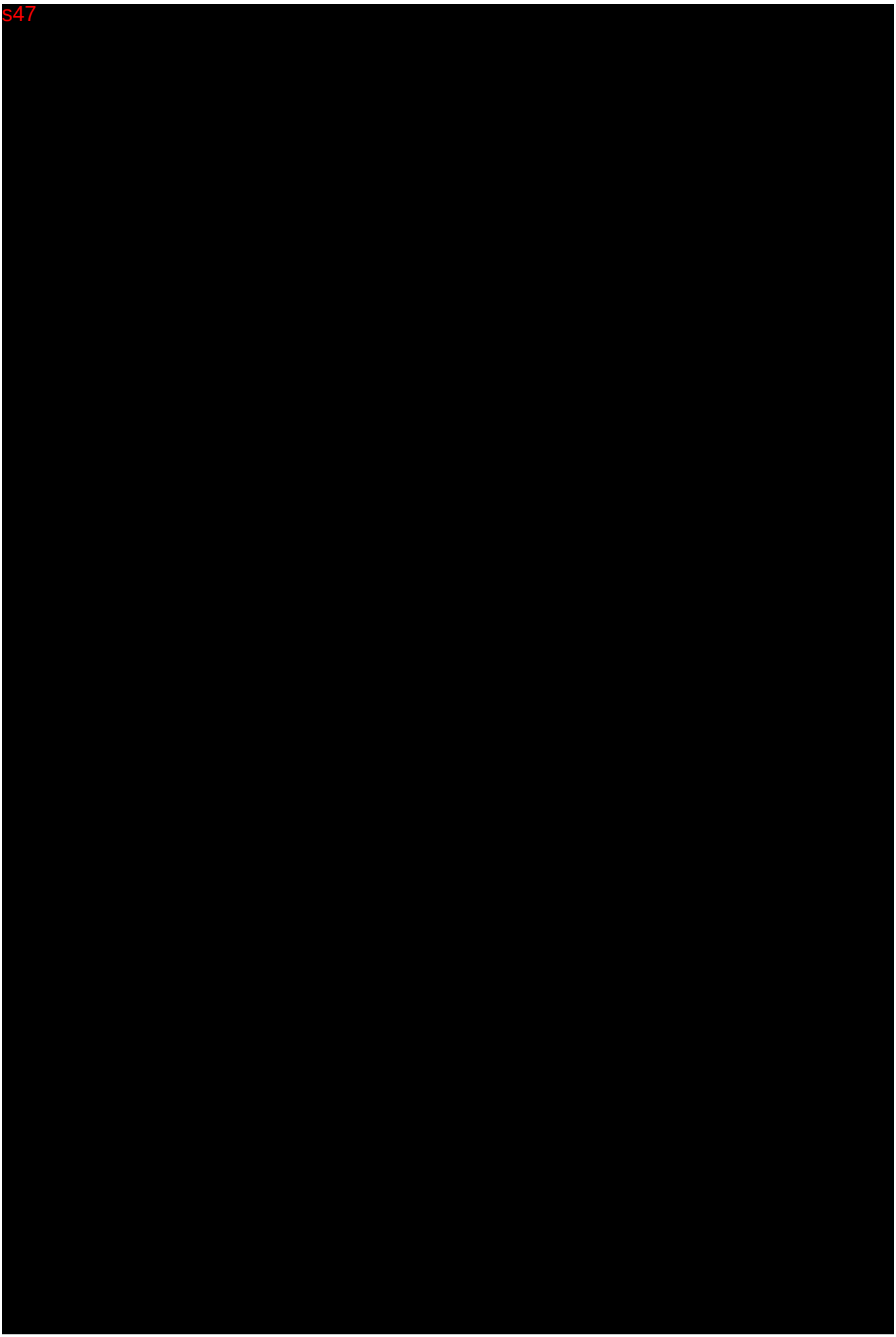

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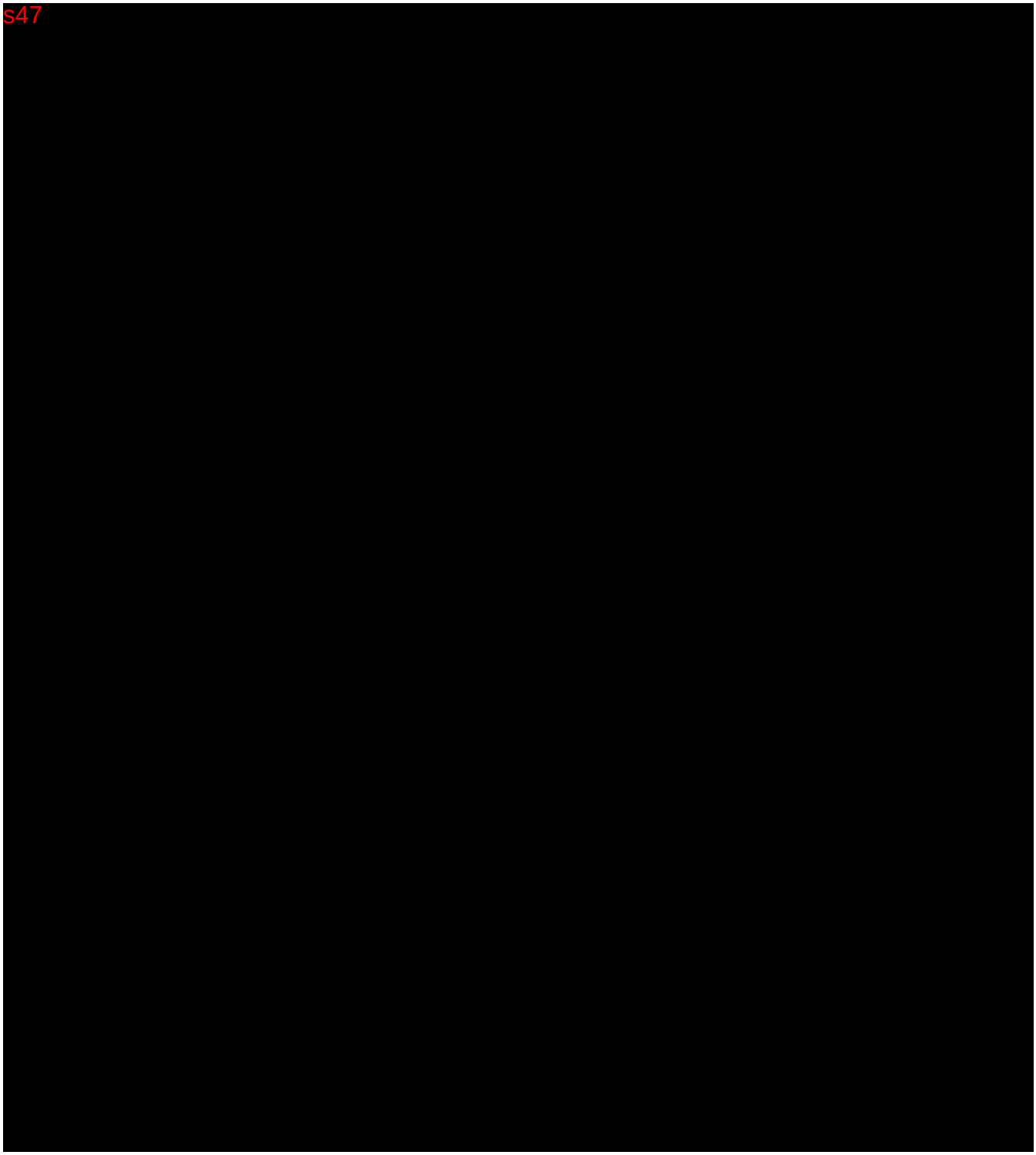

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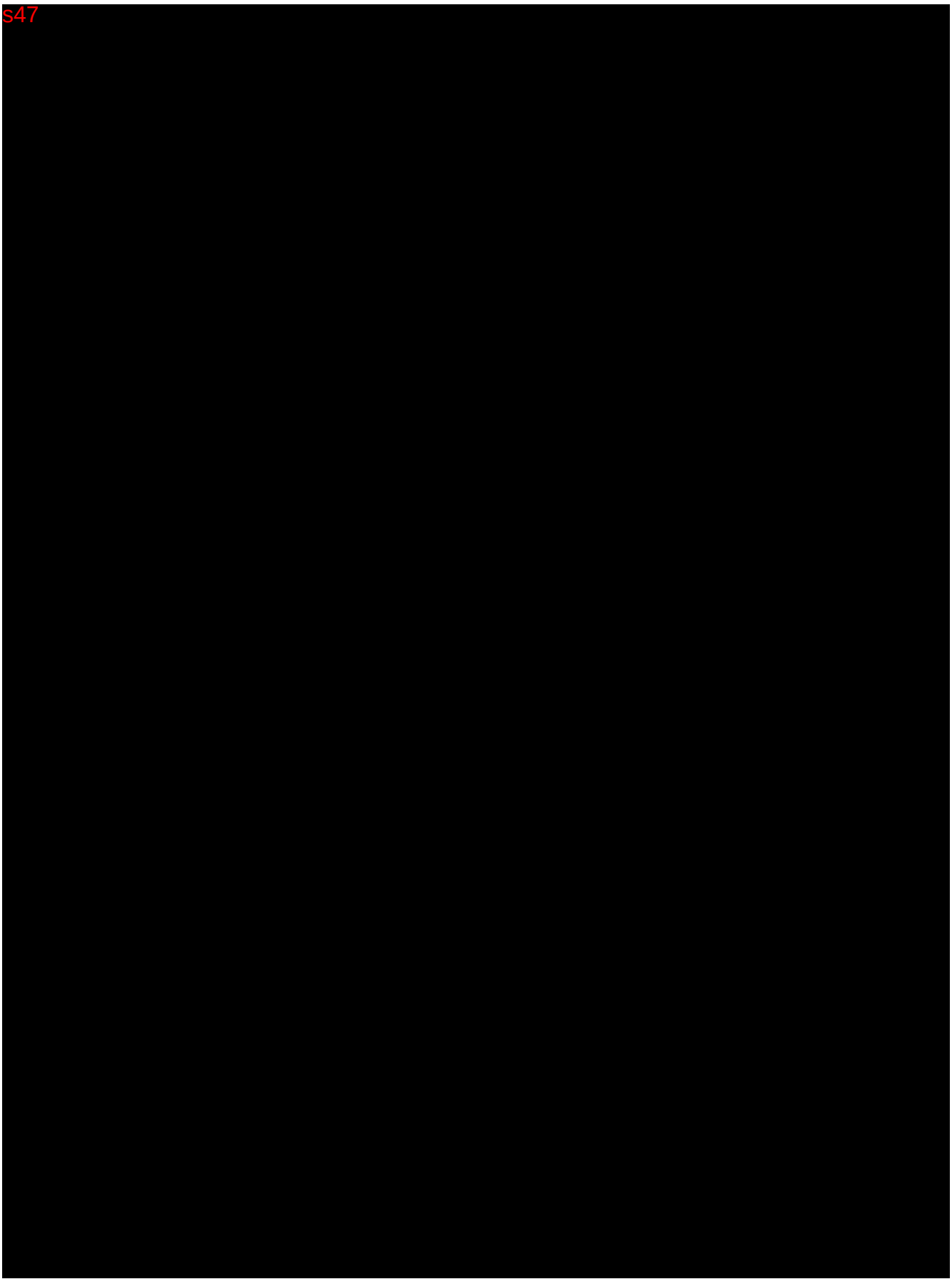

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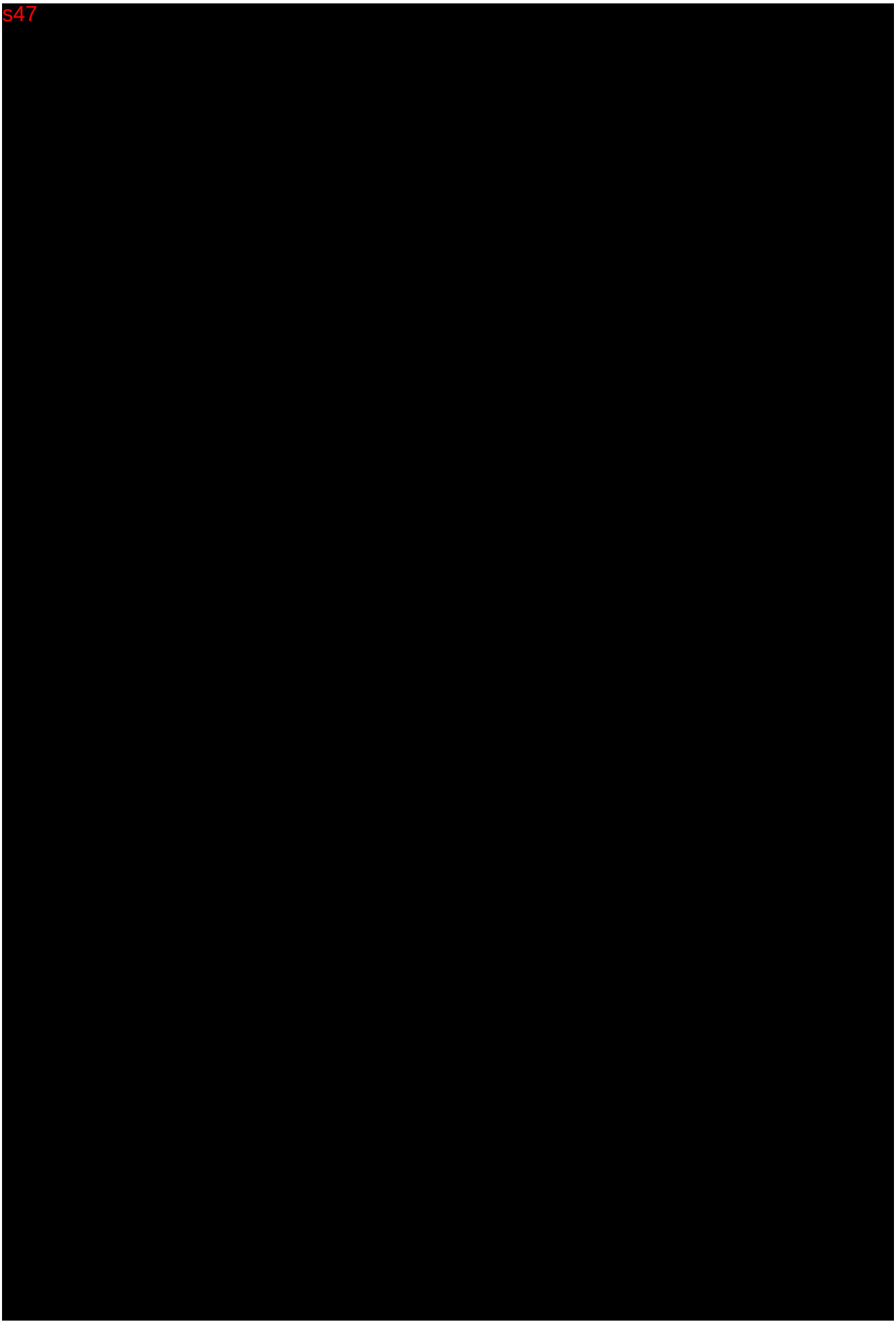

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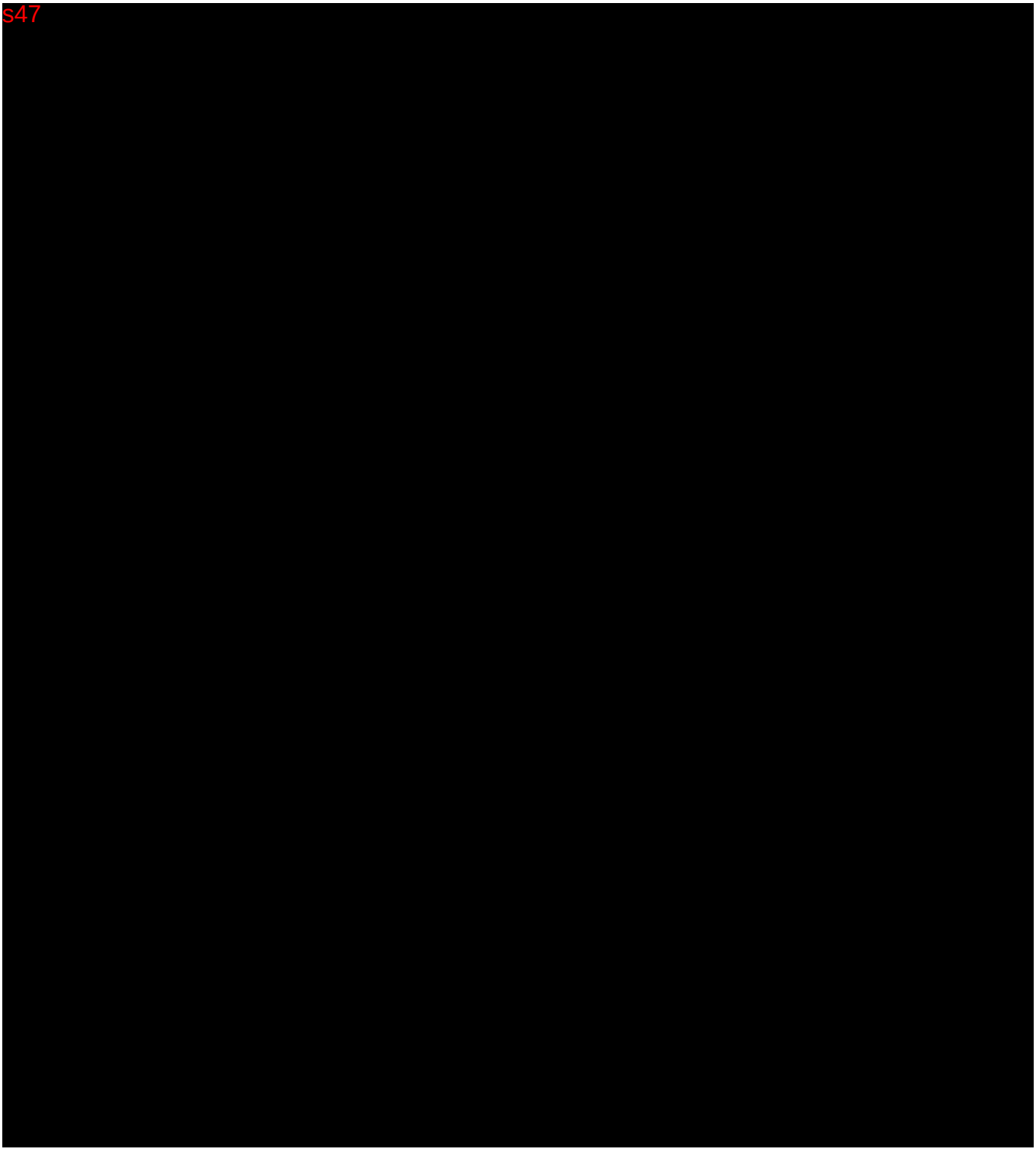

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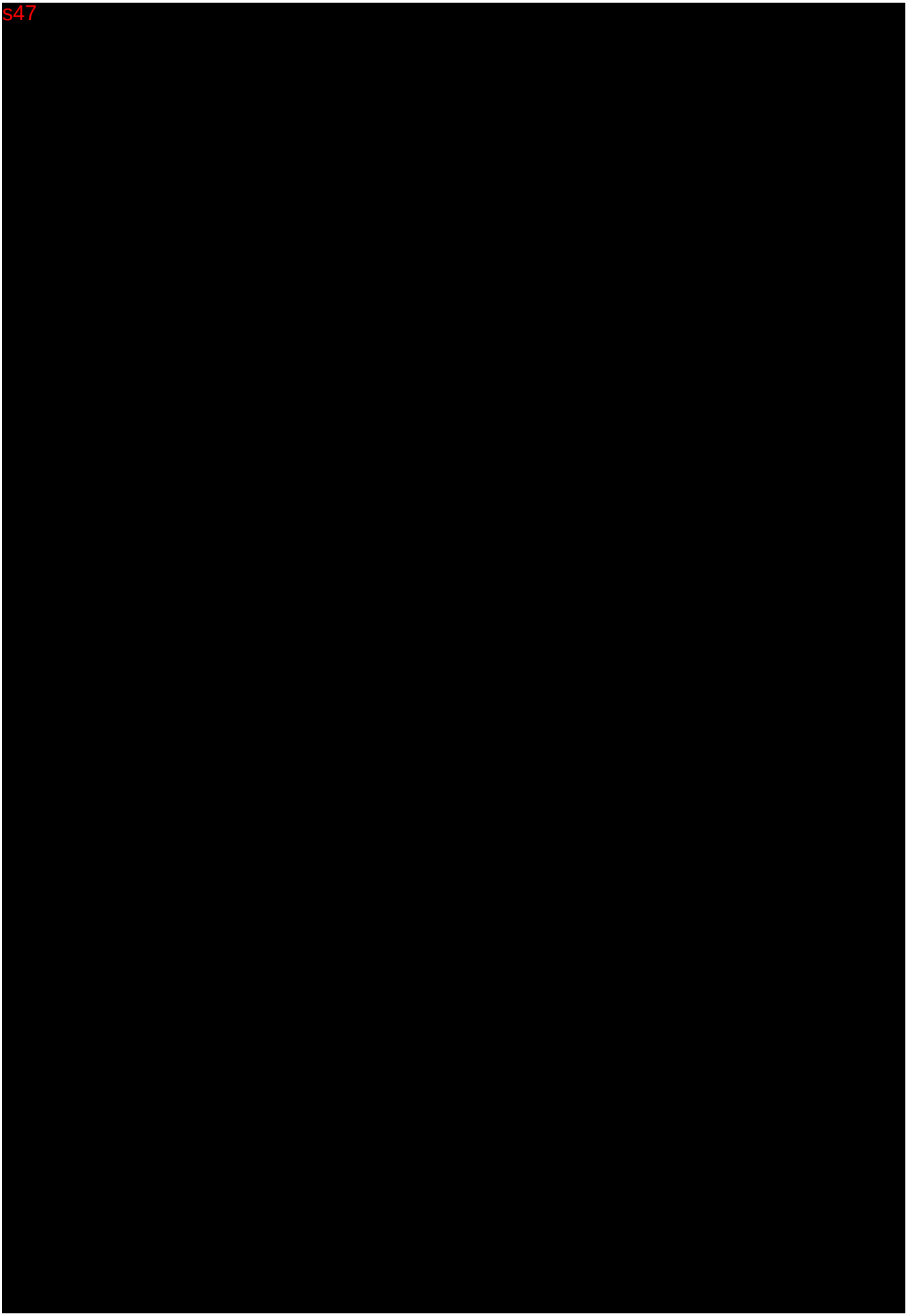

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9. GENOTOXICITY

Genotoxicity studies with selinexor are summarised in Table 9.1.

Table 9.1. Summary of genotoxicity studies

Study details	Test system & conditions	Results	Validity
Bacterial reverse mutation			
Study KS-39 (KS-0039) Charles River Laboratories Preclinical Services, Montreal, QC, Canada 30 April 2012 GLP	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E. coli* WP2 *uvr*A Preincubation & plate incorporations methods (as two separate assays, with triplicate plates); DMSO vehicle 5/6 concentrations over the range 15.8/50–5000 µg/plate ±S9	**Negative**	Yes: tested up to the recommended limit concentration; positive controls confirmed assay sensitivity
***Chromosomal aberrations* in vitro**			
Study KNC-G-13-005 (WIL-859037) WIL Research, Skokie, IL, USA 3 June 2014 GLP	Human peripheral blood lymphocytes Exposure for 3 h (±S9) or 22 h (-S9); single donor, duplicate cultures; DMSO vehicle 0.05, 0.1 & 0.5 µg/mL (3 h, -S9), 0.025, 0.05, 0.2 µg/mL (22 h, -S9), 0.1, 0.5, 2 µg/mL (3 h, +S9) 200 metaphases/concentration scored for chromosomal aberrations	**Negative**	Yes: 49–50% mitotic suppression at the highest tested concentrations; positive controls confirmed assay sensitivity
***Chromosomal aberrations* in vivo**			
Study KNC-G-13-004 (WIL-859038) WIL Research, Ashland, OH, USA 6 June 2014 GLP	Rat micronucleus test 0, 2, 4, 7, 10 mg/kg/day PO (gavage) for 3 consecutive days; vehicle: 1% poloxamer 188 & 1% povidone K-29/32 SD strain; *n* = 5/sex femoral bone marrow harvested at 18–24 h after the final dose; 2000 PCE/animal scored for micronuclei	**Negative**	Yes: high dose level was limited by severe bone marrow cytotoxicity (demonstrated in pilot experiments at ≥15 mg/kg/day); positive response demonstrated with cyclophosphamide

10. CARCINOGENICITY

No data were submitted.

11. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Embryofetal development studies were performed with selinexor in the rat.

11.1. PHARMACOKINETICS IN PREGNANT AND LACTATING ANIMALS

Placental transfer and excretion in milk were not investigated. The main rat embryofetal development study included toxicokinetic analyses.

11.2. FERTILITY AND EARLY EMBRYONIC DEVELOPMENT

No studies were submitted.

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12. LOCAL TOLERANCE

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13. OTHER TOXICITY STUDIES

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14. IMPURITIES

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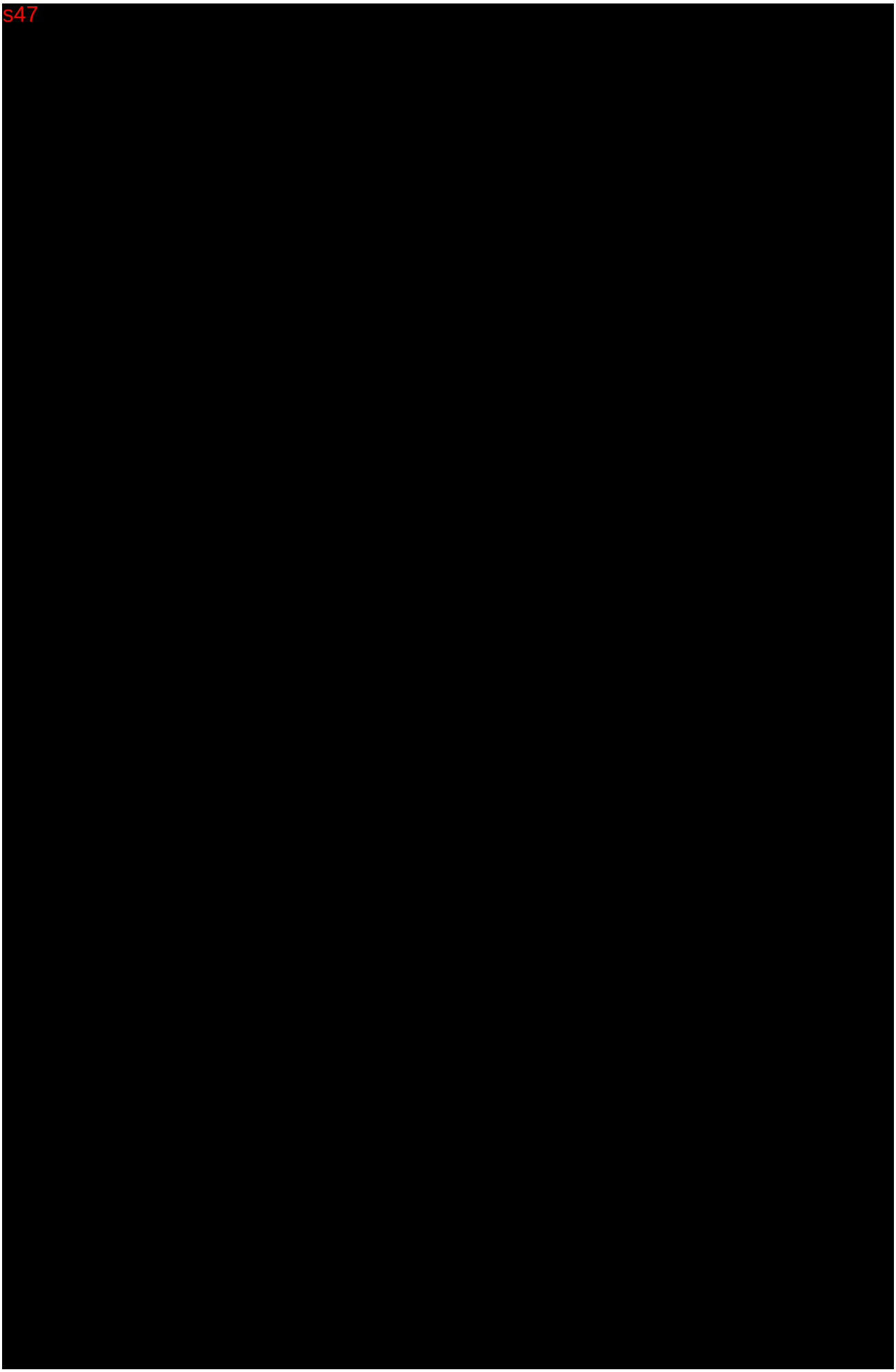

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