ORIGINAL ARTICLE

Retrospective study: Laser excision versus combined laser, cryosurgery and intralesional 5-fluorouracil in the treatment of equine sarcoids

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Summary

Background: Laser excision is used routinely in the treatment of sarcoids but may be ineffective in cases where complete excision cannot be achieved. A multimodal approach is warranted in these cases. 5-FU may improve the lethal effect of cryosurgery as an adjunct to laser excision.

Objectives: To compare two treatment protocols for equine sarcoids, laser excision alone versus a combination protocol of laser excision, cryosurgery and 5-FU chemotherapy. Factors associated with sarcoid recurrence are also investigated.

Study design: Retrospective case controlled study.

Results: Eighty-four horses with 168 histologically confirmed sarcoids were included, with a median follow-up time of 39 months (IQR 21–62 months). Sarcoid recurrence at the treated site was reported in 38% of cases and in 23% of any individual sarcoid. No significant difference was demonstrated between treatment categories in either rate of sarcoid recurrence ($p=0.45$ for any treated horse, $p=0.63$ for individual sarcoid) or time to sarcoid recurrence ($p=0.73$). Sarcoid recurrence was higher in horses with a greater number of sarcoids (OR 1.2 (1.0–1.5), $p=0.03$); when treatment had been received prior to admission (OR 7.6 (2.0–33), $p=0.004$). Horses with urogenital sarcoids or >1 mixed sarcoid experienced more rapid recurrence (HR 3.6 (1.3–10), $p=0.02$ and HR 9.9 (3.3–30), $p<0.001$) and recurrence was less rapid following the treatment of a horse's first sarcoid (HR 0.3 (0.1–0.7), $p=0.009$).

Main limitations: Significant differences in case populations in each treatment category. Treatment selection was neither blinded nor randomised and missing data and recall bias limit the study's power. Sarcoid recurrence was owner reported.

Conclusions: When assessing the likelihood of sarcoid recurrence, characteristics of both the individual patient and sarcoid phenotype must be considered carefully when selecting a specific treatment protocol.

KEYWORDS
horse, chemotherapy, cryosurgery, recurrence, sarcoid

INTRODUCTION

The equine sarcoid (ES) is the most common equine cutaneous neoplasm, responsible for approximately 46% of neoplastic cutaneous biopsy samples (Schaffer et al., 2013; Taylor & Haldorson, 2013). Although tumours are rarely metastatic, aggressive local invasion and secondary infection/ulceration may have a significant impact on equid welfare (Ireland et al., 2013; Taylor & Haldorson, 2013). Currently no uniformly effective therapy for its treatment has been reported despite publication of numerous treatment protocols (Byam-Cook et al., 2006; Carstanjen et al., 1997; Compston et al., 2016; Hollis, 2020; Hollis & Berlato, 2018; Klein et al., 1986; Knottenbelt et al., 2020; Knottenbelt & Kelly, 2000; Lane, 1977; Martens, De Moor, Vlaminck, et al., 2001; McCauley et al., 2002; McConaghy et al., 1994; Stadler et al., 2011; Stewart et al., 2006; Tamzali et al., 2012; Theon et al., 2006). Laser excision, either by CO_2 or diode laser, is widely used for the surgical removal of sarcoids (Carstanjen et al., 1997; Compston et al., 2016; Martens, De Moor, Demeulemeester, & Peelman, 2001; Martens, De Moor, Vlaminck, et al., 2001; McCauley et al., 2002). As with any surgical excision, one of the major limitations is that tumour-free margins must still be obtained and this may be difficult with equine sarcoids, depending on sarcoid type, size, number and anatomic location (Knottenbelt, 2019). Though thermal injury secondary to laser excision extends beyond the surgical field, this is variable and difficult to predict (Knottenbelt, 2019; Tate & Tate, 2019). A multimodal approach to sarcoid treatment has frequently been suggested to reduce sarcoid recurrence rates (Klein et al., 1986; Knottenbelt, 2019; Spoormakers et al., 2003; Tamzali et al., 2012), likely due to greater destruction of remaining neoplastic cells in situations where complete tumour excision has not been achieved.

Cryosurgery, the freezing of cells in this case by the application of liquid nitrogen, kills cells directly due to ice crystal formation and microcirculatory failure (Baust & Gage, 2005). Reported success rates as a sole therapy are variable (Klein et al., 1986; Knottenbelt & Kelly, 2000), with several authors reporting this therapy is most useful when used as an adjunct to other therapies, particularly in the case of larger cutaneous masses (Klein et al., 1986; Martens, De Moor, Demeulemeester, & Peelman, 2001; Martens, De Moor, Vlaminck, et al., 2001). Recent molecular research has focussed on tissues in the periphery of the cryosurgery treated zone where cell death is delayed and via apoptosis (Hollister et al., 1998). It has been suggested that further therapy promoting apoptosis in this region may be beneficial in improving the lethal effect of cryosurgery, and thereby improve outcome (Baust & Gage, 2005; Clarke et al., 1999).

5-fluorouracil (5-FU) is a fluoropyrimidine antimetabolite used in the treatment of a range of cancers, particularly breast and colorectal cancers (Longley et al., 2003). It exerts its effects via incorporation of the fluoronucleotides into cellular RNA and DNA, leading to subsequent cellular dysregulation and apoptosis (Longley et al., 2003). In horses, its use has been reported in the treatment of ocular or peri-ocular squamous cell carcinoma (Offer et al., 2022; Pucket & Gilmour, 2014), and as a topical agent for the treatment of peri-ocular sarcoids with variable success (Knottenbelt & Kelly, 2000). Intralesional 5-FU alone has a reported sarcoid resolution rate of 61.5%, though rates may be lower in large or 'resistant' tumours (Stewart et al., 2006). 5-FU is ranked amongst the safest chemotherapeutic agents (Vodenkova et al., 2020) and its use over more commonly reported platinum-based ES chemotherapy may be warranted given reduced evidence of any human carcinogenic activity (Greene, 1992; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1987). Its use as an adjunct to cryosurgery merits further investigation since it has been suggested that agents, such as 5-FU, may be beneficial in promoting the apoptotic cell death at the periphery of cryotherapeutic sites (Baust & Gage, 2005; Clarke et al., 2001). This may be particularly clinically useful in anatomic locations that limit the depth and/or extent of laser excision or cryosurgery in order to reduce the extent of damage to underlying normal tissues.

Objectives and hypotheses

This study aimed to compare two treatment protocols for the treatment of equine sarcoids: laser excision alone versus a combination protocol of laser excision, cryosurgery and 5-FU chemotherapy. As a secondary aim, factors associated with sarcoid recurrence were investigated. The authors hypothesise that the combination protocol will result in lower sarcoid recurrence rates than the laser excision protocol.

MATERIALS AND METHODS

Records were reviewed for horses referred to Glasgow Equine Hospital for the treatment of sarcoids between 2013 and 2022 (Figure 1). Criteria for case inclusion were horses with histologically confirmed sarcoids treated either by laser excision via diode laser or by the below combination therapy protocol. Cases were excluded if histopathological confirmation was not available, treatment was incomplete, or if a combination of laser excision/combination therapy/ any other combination treatment was employed.

Excision via diode laser consisted of use of the laser on continuous mode and a power setting of between 15 and 25 W, at the surgeon's discretion. Adherence to laser safety protocols was maintained. Where possible, the surgeon achieved a margin of at least 1 cm of grossly normal tissue around the sarcoid, although this was limited in cases where masses were very large and/or infiltrative, depending on their anatomic location. In this protocol, surgical sites were either left to heal by secondary intention or were closed using a 'smart' surgical technique, as previously described (Knottenbelt et al., 2015). When using the combination protocol, laser excision sites were left open, and excision margins were then treated with three freeze–thaw cycles of cryosurgery followed by administration of 5-FU solution into the margins. Cryosurgery was performed using the CryoPro® cryosurgery unit (Cortex Technology, Denmark). The unit was held approximately
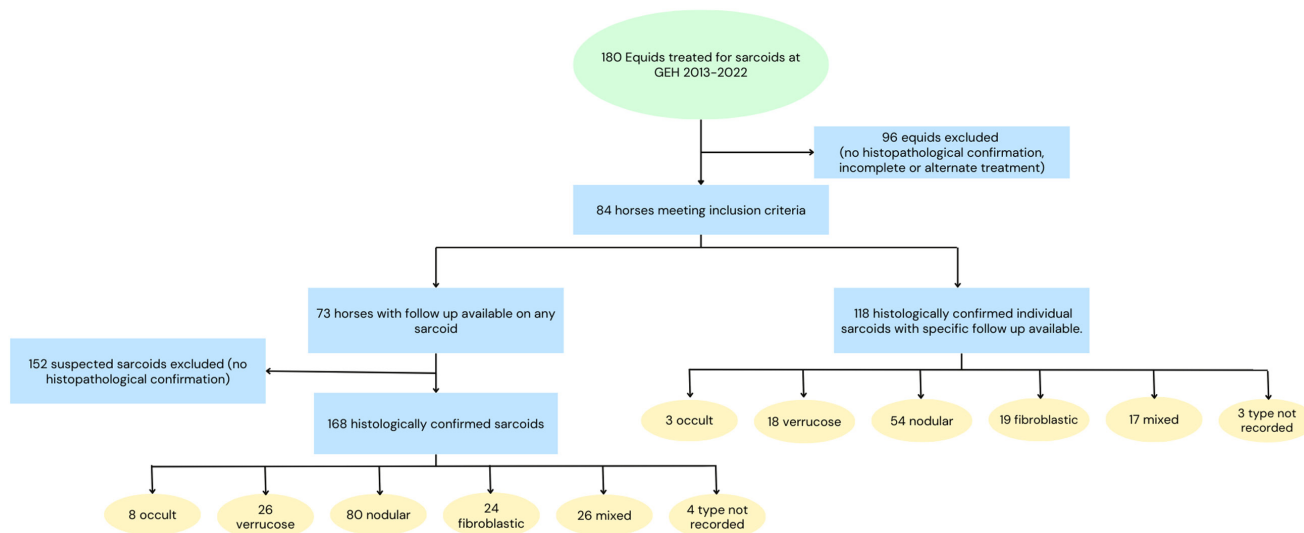
FIGURE 1 Flow chart describing cases and sarcoids included in the retrospective analysis.

10 cm from the desired site and liquid nitrogen was applied to spray freeze the epidermal margins and exposed dermis until firm. Care was taken to avoid excessive freezing of the underlying tissues. The tissues were allowed to thaw, before repeating a further two times. Thereafter, 50 mg/mL 5-FU (Medac) was injected into the resultant wheal to an estimated dose of 1 mL/cm^3 target tissue. Appropriate personal protective equipment (two layers of chemotherapy-approved nitrile gloves, full length gown and face shield) was used during the application, and the horse handled only when wearing suitable nitrile gloves for 3 days thereafter. The horse was re-examined between 2 and 5 weeks thereafter, and the cryosurgery and 5-FU application repeated. This was repeated as required by clinical progress, for a median of three total treatments (IQR 2–3). The decision to repeat cryosurgery and chemotherapy after the three treatments was based on clinical assessment of the appearance of the granulation bed, with nodular growth a potential indicator of remaining sarcoid tissue.

Data obtained from medical records included: case history and signalment, details of the lesion(s) (sarcoid type, location, number, estimated tumour dimensions and volume), treatment protocol, associated adverse effects and duration, response to treatment and sarcoid regrowth/new lesion occurrence. The total number of suspected sarcoids, including unconfirmed and confirmed masses, was recorded for each case, but for all further analysis only histologically confirmed masses were included.

For the purpose of recording sarcoid location, the body was split into seven anatomic regions: peri-ocular, head and neck, dorsum (above the level of the olecranon process), ventrum (below the level of the olecranon process), upper limb (above the level of the tarsocrural/radiocarpal joint), lower limb (below the level of the tarsocrural/radiocarpal joint) and urogenital (affecting the prepuce, penis, mammary glands or vulva). Sarcoid type was recorded using the currently accepted convention (Knottenbelt, 2005). Previous histopathological findings were recorded, including the presence or absence of confirmed surgical margins.

Following initial correspondence, owners were contacted via telephone between August and October 2022 to determine treatment outcome. An example of the telephone questionnaire is included in Questionnaire S1. Data were collected regarding the horse's history of sarcoid growth and treatment, outcome following sarcoid treatment, regrowth of treated sarcoids and growth of any new sarcoids.

Data analysis

Data were analysed both by case, and then by individual sarcoid. Statistical analysis was performed in R studio (R version 4.2.0) (R Core Team, 2022). Normality was assessed for continuous variables using the Shapiro–Wilk test. Thereafter, as data were nonparametric, univariate analysis was performed by chi-squared test or Fisher's exact test for categorical variables, Wilcoxon's rank sum for continuous variables and Cochrane Armitage test (two sided) for trend, where appropriate (Table S1).

For variables of $p \leq 0.2$ in the univariate analysis, logistic regression was performed with sarcoid recurrence as the dependent variable (Table S2). Variables with $p \leq 0.2$ in the univariate analysis, $n > 10$ and low collinearity with other independent variables, were used to perform multiple logistic regression, again with recurrence as the control-dependent variable. Variables were removed from the model in a manual backwards elimination manner with the Akaike information criterion (AIC) used to assess the fit of the model at each step. Variables were retained in the final model despite not fulfilling $p \leq 0.2$ when their inclusion improved the AIC of the model. Variance inflation factors were used to assess final variables for multicollinearity, binned residual plots created to assess normality of the residuals and a receiver operating curve constructed in order to assess the adequacy of the model.

All variables with $p \leq 0.2$ in the univariate logistic regression were evaluated for inclusion in a Cox's proportional hazards model. Data

were right censored at either time to recurrence of the treated sarcoid, time to follow-up telephone conversation or time to death or sale of the horse. Where multicollinearity existed, the value with the lowest p-value was included. Numerical covariates were transformed to categorical due to violation of the linearity assumption of the Cox's proportional hazards model. The model was refined using a manual backwards elimination approach, with threshold for inclusion in the model at $p \leq 0.2$ whilst maximising concordance of the model. A plot of scaled Shoenfeld residuals was used to assess the proportional hazards assumptions of the model. Time to sarcoid recurrence was used to create a Kaplan–Meier plot, and differences between treatment protocols compared with a log-rank test. Statistical significance was considered at $p < 0.05$.

RESULTS

One hundred and eighty equids were referred to Glasgow Equine Hospital for the treatment of sarcoids during this period, of which 84 met the inclusion criteria. Histological confirmation was available for 168 individual sarcoids in these 84 horses. Of these, 8 (5%) were categorised clinically as occult sarcoids, 26 (15%) as verrucose, 80 (48%) as nodular, 24 (14%) as fibroblastic and 26 (15%) as mixed. None were classed as 'malevolent', and sarcoid type was not recorded in four cases (2%). Seventeen different breeds were represented, of which 17 horses were crossbreeds (20%), 11 Irish Sport Horses (13%), 10 warmbloods (12%), six Thoroughbreds (7%) and five cobs (6%). Four donkeys were included in the study (5%), and the remaining horses were of various breeds. Thirty-two of included equids were mares (38%), 51 geldings (61%) and 1 stallion (1%). Ages ranged from 2 to 20 years (median 8 years (IQR 6–14 years)). Owner follow-up was available for 69/84 individuals, with regrowth ascertained from medical records in a further four individuals.

A flow chart describing included cases and sarcoids is presented in Figure 1. Of the 73 cases for which follow-up was available, 168 of the total of 320 suspected sarcoid masses were histologically confirmed (53%) and were included in further analysis. Specific follow-up regarding individual sarcoid regrowth was available for 118 histologically confirmed masses.

Horses/sarcoids for which follow-up was not available were excluded from further analysis regarding sarcoid regrowth. 62 horses had multiple sarcoids at the time of presentation, with a median of 3 (IQR 2–7). 32 of these horses had ≥5 sarcoids, 15 ≥ 10 sarcoids and 3 horses had ≥20 sarcoids. All masses were removed at the time of initial presentation, with a maximum number 24 suspected sarcoids.

During the study period, the overall incidence of recurrence of any sarcoid on an equid at the same previously treated site was 38% (28/73) and time to follow-up ranged from 0.75 to 132 months (median 39 months (IQR 21–62 months)). For any individual treated sarcoid, total rate of recurrence was 23% (29/128) with a median length of follow-up of 48 months (IQR 24–61 months).

In total, 28 equids were treated by the combination treatment protocol (33%) and 56 by diode laser excision alone (67%). Table 1 displays pertinent differences between treatment groups. Equids in the combination category had larger sarcoids (median width 59 vs. 30 mm, $p < 0.001$) and were less likely to have any incision closed at the time of excision ($p < 0.001$). Length of follow-up was significantly longer in the laser category (52 months (39–72 months) vs. 20 months (14–25 months) $p < 0.001$).

Complications following treatment occurred in 14 horses included in the study (17%). These were more frequent in horses treated by combination protocol (9/25 (36%) vs. 5/45 (11%), $p = 0.03$) but were short lived and comprised: oedema surrounding the treated site (5/14, 36%), owner reported delayed healing (3/14, 21%) or infection (3/14, 21%) of the treated site, and individual cases of myiasis, facial nerve neuropraxia and excessive scarring (1/14, 7% each). Overall, 60/67 owners (90%) said they were happy with the final cosmetic result of the treatment. Of the 16 horses reported to have been euthanised prior to follow-up, four of these were for sarcoid-related reasons, resulting in a fatality rate of 4/69 (5.8%) over the duration of the follow-up period in this referral population.

Sarcoid recurrence at any treated site was reported in 20/47 (43%) of horses receiving laser excision alone and 8/26 (31%) treated by combination therapy for which follow-up was available. For any individual sarcoid, eventual recurrence at the same site occurred in 23/95 (24%) of those treated by laser excision and 6/33 (18%) receiving the combination protocol. These differences were not statistically significant in either case ($p = 0.32$ and $p = 0.48$ respectively).

Likelihood of sarcoid recurrence

A forest plot of variables significant in the univariate logistic regression on both a whole case and individual sarcoid basis is shown in Figure 2. Full results are available in Table S1.

Univariate logistic regression indicated that recurrence was more likely if the ventrum was affected (OR 3.9 (1.5–11) $p < 0.001$), and if sarcoids were of verrucose (OR 1.9 (1.2–3.4), $p = 0.01$) or mixed (OR 1.9 (1.3–3.5), $p = 0.002$) types. 19 horses and 20 individual sarcoids had received attempted sarcoid treatment prior to initial presentation, and this was significantly associated with subsequent sarcoid recurrence (13/19 (68%), OR 7.7 (2.5–27) $p < 0.001$ and 9/20 (45%), OR 4.9 (1.5–16) $p = 0.004$). Having a greater total number of sarcoids at presentation was also associated with recurrence (OR 1.3 (1.1–1.5), $p < 0.001$) as was the surgical closure of any incision at the time of initial treatment (OR 4.0 (1.5–11.6), $p = 0.007$). Sarcoid chronicity was associated with recurrence both for affected animals (cases) and individual sarcoids ($p = 0.03$ and <0.001) and any individual sarcoid treated under general anaesthesia was more likely to recur (OR 6.3 (2.5–17) $p < 0.001$) than if treated standing under sedation. A significant association was also demonstrated between chronicity and sarcoid width ($p = 0.02$, OR 1.01 (1.00–1.01)) and the likelihood of attaining histological margins

TABLE 1 Pertinent patient and sarcoid characteristics of cases assigned to either treatment category and results of univariate comparison between treatment categories.

	Laser	Combination	p	N
Age (years)	8 [6–10.75]	8.5 [7–10.8]	0.32	84
Site(s) affected				
Periocular	12 (25%)	2 (7%)	0.13	14
Head and neck	15 (27%)	7 (25%)	0.86	22
Dorsum	2 (4%)	0 (0%)	0.55	2
Ventrum	27 (48%)	19 (64%)	0.09	46
Upper limb	33 (59%)	19 (64%)	0.43	52
Lower limb	2 (4%)	0 (0%)	0.55	2
Urogenital	14 (25%)	4 (14%)	0.40	15
Chronicity at presentation				
<3 months	16 (35%)	6 (26%)	0.69	22
3–6 months	7 (15%)	4 (17%)		11
6–12 months	6 (13%)	5 (22%)		11
>12 months	17 (37%)	8 (35%)		25
Total number of sarcoids	3 [1–7.25]	3 [2–6.5]	0.53	83
Max sarcoid width (mm)	30 [15–50]	59 [40–95]	<0.001*	64
Histological margins confirmed clear	10 (18%)	5 (18%)	0.75	84
≥1 Wound closed	27 (48%)	2 (7%)	<0.001*	84
Restraint				
Standing sedation	28 (50%)	20 (71%)	0.06	48
GA	28 (50%)	8 (29%)		36
Complications	5 (11%)	9 (36%)	0.03*	70
Length of follow-up (months)	52 [39–72]	20 [14–25]	<0.001*	72

Note: A total number of 84 cases were included in the study, but N is variable as full data were not available for every criterion assessed here and so the relevant field size is given in each case. *denotes statistical significance.
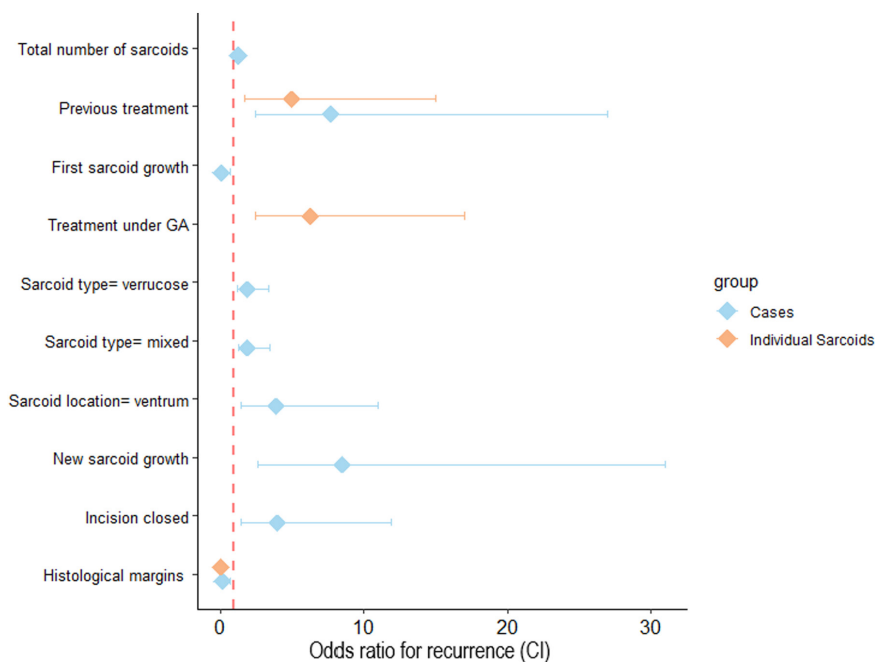

FIGURE 2 Forest plot showing odds ratios (OR) and 95% confidence intervals for variables significantly associated with likelihood of sarcoid recurrence within an individual case and for individual treated sarcoid lesion. The red dashed line indicates an OR of 1.

TABLE 2 Multiple logistic regression models for variables associated with sarcoid recurrence in 84 horses with histopathological confirmation of sarcoid(s).

	Coefficient	SE	Wald's test statistic	p	OR [CI]	AIC
Intercept	−2.2	0.68	−3.3	0.001*	0.11 [0.02–0.36]	60
Total number sarcoids	0.19	0.09	2.1	0.03*	1.2 [1.0–1.5]	
Histological margins achieved	−0.90	0.95	−0.96	0.34	0.40 [0.05–2.3]	
Treatment prior to presentation	2.0	0.71	2.9	0.004*	7.6 [2.0–33]	

Note: *denotes statistical significance.

TABLE 3 Cox's proportional hazards model of variables associated with time to sarcoid recurrence showing hazard ratios (HR) and 95% confidence intervals [CI] for included variables.

	HR [CI]	p	Concordance
Lower limb	0.2 [0–1.6]	0.13	0.75
Urogenital	3.6 [1.3–10]	0.02*	
Number mixed sarcoids		<0.001*	
0	Reference		
1	3.5 [1.0–12]	0.046*	
>1	9.9 [3.3–30]	<0.001*	
First sarcoid	0.30 [0.10–0.70]	0.009*	

Note: *denotes statistical significance.

($p=0.01$, OR=0.68 (0.50–0.92)). Neither sarcoid site nor width was significantly associated with the confirmation of histological margins ($p=0.99$ and $p=0.15$, respectively).

Table 2 displays the multiple logistic regression model for variables predictive of sarcoid recurrence in any presenting case. The area under the ROC curve for this model as a predictor of sarcoid recurrence in this population was 0.86. A mixed effects logistic regression model was attempted with 'equid' included as the random effect variable. The random effect of horse-related factors was shown to be extremely large (between subject variance=1065) and so this model was rejected due to clustering of data. Two horses were over-represented in the individual sarcoid data, these being 6- and 7-year-old Warmblood geldings with specific follow-up available on eight and seven sarcoids, respectively. Both geldings were treated by laser excision alone and only two sarcoids recurred.

The effect of treatment category on recurrence rate whilst accounting for significant population differences between treatment category (largest sarcoid width (mm), closure of any incision, occurrence of complications following treatment and length of case follow-up (days)) was examined. Treatment category remained non-significant in this model (OR 1.3 (0.14–13), $p=0.82$).

Time to sarcoid recurrence

Data regarding time to sarcoid recurrence were available for 72 horses. Variables retained as significantly associated with time to sarcoid recurrence in the Cox's Proportional Hazards model are presented in Table 3, and the final Kaplan–Meier plot of sarcoid

recurrence for each treatment category is presented in Figure 3. The log-rank test comparing the survival curves again indicated no significant difference between treatment categories ($p=0.73$).

DISCUSSION

Treatment protocols

The rate of recurrence of any sarcoid in this study was 23%, with a median length of follow-up of 48 months. There was no significant difference in recurrence between treatment category (laser 24% (23/95), combination 18% (6/33)). The lack of routinely accepted standardised outcome measure for the treatment of sarcoids makes comparison between studies very difficult (Offer et al., 2024). Though these recurrence rates are marginally higher than those reported in the literature (sarcoid regression rates of 83%–89% with sole laser excision) (Compston et al., 2016; Martens, De Moor, Demeulemeester, & Peelman, 2001; Martens, De Moor, Vlaminck, et al., 2001), this is highly likely to be influenced by numerous factors, including greater length of follow-up period in this study, and differences in case selection and treatment populations. These horses were all referred to the hospital for further evaluation and treatment, and by definition were positioned at the more severe end of sarcoid phenotype presentation.

Employing a multimodal approach to the treatment of sarcoids has been suggested by previous authors as a method to improve sarcoid regression rate (Knottenbelt, 2019) and was highlighted by this author's recent systematic review as likely to be advantageous
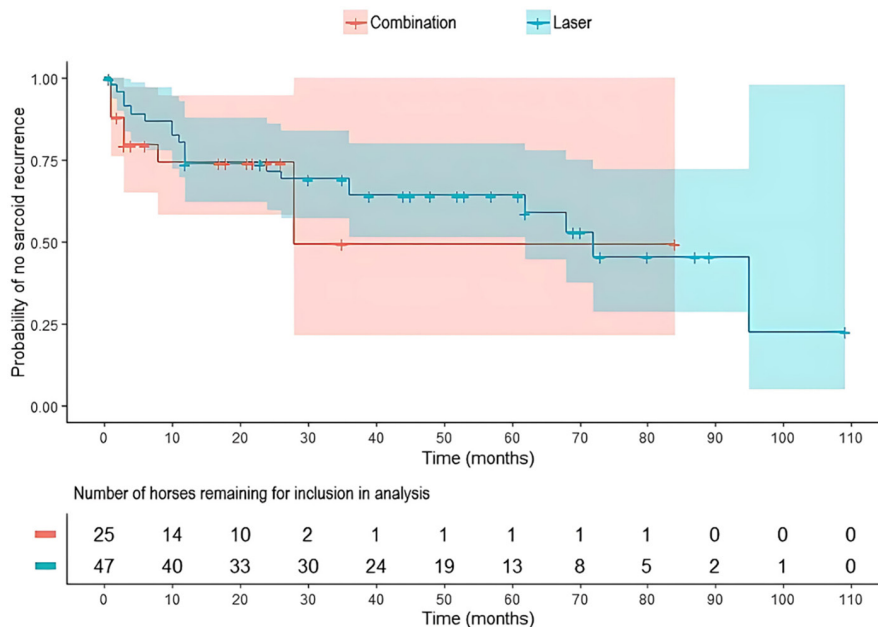
FIGURE 3 Kaplan–Meier plot showing time to sarcoid recurrence in both treatment categories. The table shows the number of included horses remaining with no sarcoid recurrence at each time point and in each treatment group, and therefore remaining for inclusion in analysis.

over single treatment modalities (Offer et al., 2024). This was not demonstrated in this study, though several confounding factors exist that may have prevented this. Firstly, there exist several significant differences in the case populations between treatment groups. Sarcoids treated by the combination protocol were significantly larger ($p < 0.001$) and were less likely to have been treated under general anaesthesia ($p = 0.04$). Both these factors may prevent attainment of an adequate surgical margin, depending on the location of the sarcoid and the temperament of the horse. As this study was not a randomised prospective trial, clinician preference is also likely to have resulted in selection of the combination protocol for sarcoid masses of greater clinical severity. Sarcoids in the combination treatment category were also on significantly older horses. Whilst rare, spontaneous remission does occasionally occur in younger horses, and so this may have further confounded the results (Berruex et al., 2016). Conversely, equids treated by laser therapy alone had a significantly longer time to follow-up than those treated by the combination protocol (median 52 vs. 20 months) due to the distribution of cases and historical clinician preference. Longer-term recurrence may therefore be underestimated in the combination protocol treatment category.

However, when a multivariable model was constructed to account for differences in case population between categories, treatment category remained nonsignificant in the model ($p = 0.82$). Since sarcoid recurrence is dependent on numerous variables (Knottenbelt, 2019), case selection is crucial in determining its likelihood. A true comparison of these treatment techniques would therefore require a fully randomised and blinded controlled trial and a recommendation between these two treatment protocols cannot be made on the basis of this study.

Cryosurgery and cryobiology are rapidly evolving fields of cancer therapy. Current recommendations for cryosurgical treatment of neoplasms include achieving a minimum temperature of −40°C for at least 5 min, and then allowing the thaw to be as slow as is practicable (Baust & Gage, 2005; Hoffmann & Bischof, 2002). Such a technique aims to produce a central zone of coagulative necrosis and direct cell death, as discussed previously, and requires the direct monitoring of freezing temperatures either by a needle mounted temperature probe or indirectly ultrasonographic changes. Freezing temperatures in this protocol were monitored only clinically, with the manual palpation of a direct zone of freezing. The use of multiple freeze–thaw cycles was designed to increase the cellular physiochemical changes occurring in neoplastic cells and is employed in several cancers as a technique to increase the lethal freezing temperature required and extend the zone of necrosis to closer to the tumour margins without endangering underlying tissues. Despite this, it is unlikely that the published target of −40°C was achieved, and indeed may have been undesirable given the expected damage to underlying tissues with such low temperatures.

In this clinical situation, the secondary inflammatory effects and delayed cellular apoptosis following cryosurgery become more significant, giving further weight to the use of an adjunctive proapoptotic agent such as 5-FU. Overcoming the immune evasive micro-environment of sarcoids is increasingly accepted to be crucial in the treatment of this disease (Jindra et al., 2023), and so cryosurgery in this instance may represent a method of nonspecific immune stimulation and theoretically contribute to tumour resolution.

Risk factors for recurrence

The total number of sarcoids on the individual at the time of presentation was significant in both the univariate and multivariate logistic

regression. This supports increased susceptibility of certain individuals to the development of sarcoids and was confirmed by the large inter-equid variance indicated in the attempted mixed effects logistic regression. This is well recorded in the literature—the presence of multiple sarcoids has been reported by several authors as predictive of an increased likelihood of sarcoid recurrence (Compston et al., 2016; Lane, 1977), and it has been shown by numerous authors that the susceptibility to sarcoids has at least some genetic basis (Broström et al., 1988; Christen et al., 2014; Lazary et al., 1994). The presence of equine leucocyte antigen (ELA) W13 allele has been correlated with susceptibility to sarcoid growth, although horses without the allele may also develop sarcoids (Goodrich et al., 1998). Candidate genes within specific chromosomal regions also have been associated with increased susceptibility to sarcoid growth (Jandova et al., 2012), and a polygenic mode of inheritance with 21% heritability has been demonstrated (Christen et al., 2014). Presentation at the time of first sarcoid growth was similarly retained in the survival analysis as predictive of an increased time to subsequent sarcoid regrowth, that is, those individuals with increased susceptibility to sarcoid growth who present with multiple sarcoids spanning several years have a shorter time to regrowth than those with solitary sarcoids at first presentation.

Treatment of any sarcoid prior to presentation was associated with sarcoid recurrence and was retained in the multivariable model. The reason for this may be twofold; presentation following prior treatment indicates that any previous intervention has failed to resolve the sarcoid. The sarcoid itself is therefore likely to be highly locally invasive and/or anatomically difficult to remove in its entirety. In addition, any traumatic intervention to a sarcoid may cause accelerated growth or increased malignancy, leading to subsequent difficulty in achieving full sarcoid resolution (Knottenbelt, 2019). Similarly, recurring sarcoids are likely to be more aggressive and infiltrative than those treated on first presentation (Taylor & Haldorson, 2013).

Further variables associated with sarcoid recurrence in this study may be associated with the ability to achieve surgical margins at initial laser excision. Sarcoid chronicity and size were significantly associated, and both likely to be linked to the ability to achieve complete tumour excision via laser. Complete excision of neoplastic cells logically is often stated as a significant factor contributing to sarcoid resolution (Carstanjen et al., 1997; Knottenbelt, 2019), though the desirable surgical margins for equine sarcoids have not been well defined. A margin of between 1 and 2 cm is often stated as desirable as BPV DNA has been demonstrated within a surgical margin of 16 mm in 33% of cases (Compston et al., 2016; Knottenbelt, 2019; Martens, De Moor, Demeulemeester, & Peelman, 2001; Martens, De Moor, Vlaminck, et al., 2001) and histopathological evidence of sarcoid infiltration has been demonstrated at 2 cm from the sarcoid margin removed via laser excision (Mair & Fews, 2016). Coagulative necrosis extends beyond the surgical site when laser excision is employed but this may be insufficient to prevent sarcoid recurrence in cases where neoplastic cells extend beyond this region of necrosis (Compston et al., 2016; Knottenbelt & Kelly, 2000). However, the presence of papillomaviral DNA within the surgical margin has inconsistently been associated with sarcoid recurrence, as has any association between width of surgical margin and subsequent recurrence rate (Martens, De Moor, Demeulemeester, & Peelman, 2001; Martens, De Moor, Vlaminck, et al., 2001).

Limitations

There are a number of further limitations to this study. Firstly, as with any retrospective study, missing data and loss of cases to follow-up limits its power. Owner recall bias may skew results, though answers were corroborated with written clinical records where possible. The data described here apply only to a referral hospital with one case population in which most equids had more than one sarcoid and may have undergone previous treatment attempts. With the treatment of equine sarcoids, case selection plays a significant role in the likelihood of tumour recurrence. Though populations in each treatment group were compared, they were not identical and treatment assignation was neither randomised nor blinded. Similarly, there was no untreated or placebo control population in this study, and so findings should be interpreted with caution. A significant limitation with the survival analysis was the differing follow-up times between treatment categories, although it should be noted that both exceeded the 12-month period used in many previous studies. This combination of factors may have prevented any demonstration of significant differences in time to sarcoid regrowth between groups.

CONCLUSION

No significant difference in sarcoid recurrence rate or time to sarcoid recurrence has been demonstrated with the use of a cryosurgery/chemotherapy protocol following laser excision versus laser excision alone, though this is limited by available time to follow-up and differences in case selection. The characteristics of the patient and sarcoid(s) were demonstrated to be more significant than the addition of adjunctive cryosurgery/chemotherapy in this population in relation to subsequent recurrence. Regardless of treatment protocol, approximately 25% sarcoid recurrence may be expected at 24 months following hospital discharge in a referral population of this type.

CONFLICT OF INTEREST STATEMENT
The authors have no competing interests to declare.

ETHICS STATEMENT
Ethical approval was obtained from the University of Glasgow Research Ethics Committee (reference EA42/21).

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REFERENCES
Baust, J.G. & Gage, A.A. (2005) The molecular basis of cryosurgery. *BJU International*, 95(9), 1187–1191. Available from: https://doi.org/10.1111/j.1464-410X.2005.05502.x

Berruex, F., Gerber, V., Wohlfender, F.D., Burger, D. & Koch, C. (2016) Clinical course of sarcoids in 61 Franches–Montagnes horses over a 5–7 year period. *Veterinary Quarterly*, 36(4), 189–196. Available from: https://doi.org/10.1080/01652176.2016.1204483

Broström, H., Fahlbrink, E., Dubath, M.L. & Lazary, S. (1988) Association between equine leucocyte antigens (ELA) and equine sarcoid tumors in the population of Swedish halfbreds and some of their families. *Veterinary Immunology and Immunopathology*, 19(3–4), 215–223. Available from: https://doi.org/10.1016/0165-2427(88)90109-2

Byam-Cook, K.L., Henson, F.M.D. & Slater, J.D. (2006) Treatment of periocular and non-ocular sarcoids in 18 horses by interstitial brachytherapy with iridium-192. *Veterinary Record*, 159(11), 337–341. Available from: https://doi.org/10.1136/vr.159.11.337

Carstanjen, B., Jordan, P. & Lepage, O.M. (1997) Carbon dioxide laser as a surgical instrument for sarcoid therapy – a retrospective study on 60 cases. *Canadian Veterinary Journal*, 38(12), 773–776.

Christen, G., Gerber, V., Dolf, G., Burger, D. & Koch, C. (2014) Inheritance of equine sarcoid disease in Franches–Montagnes horses. *Veterinary Journal*, 199(1), 68–71. Available from: https://doi.org/10.1016/j.tvjl.2013.09.053

Clarke, D.M., Baust, J.M., Van Buskirk, R.G. & Baust, J.G. (2001) Chemo-cryo combination therapy: an adjunctive model for the treatment of prostate cancer. *Cryobiology*, 42(4), 274–285. Available from: https://doi.org/10.1006/cryo.2001.2333

Clarke, D.M., Hollister, W.R., Baust, J.G. & Van Buskirk, R.G. (1999) Cryosurgical modeling: sequence of freezing and cytotoxic agent application affects cell death. *Molecular Urology*, 3(1), 25–31.

Compston, P.C., Turner, T., Wylie, C.E. & Payne, R.J. (2016) Laser surgery as a treatment for histologically confirmed sarcoids in the horse. *Equine Veterinary Journal*, 48(4), 451–456. Available from: https://doi.org/10.1111/evj.12456

Goodrich, L., Gerber, H., Marti, E. & Antczak, D.F. (1998) Equine sarcoids. *Veterinary Clinics of North America: Equine Practice*, 14(3), 607–623, vii. Available from: https://doi.org/10.1016/s0749-0739(17)30189-x

Greene, M.H. (1992) Is cisplatin a human carcinogen? *Journal of the National Cancer Institute*, 48(5), 306–312. Available from: https://doi.org/10.1093/jnci/84.5.306

Hoffmann, N.E. & Bischof, J.C. (2002) The cryobiology of cryosurgical injury. *Urology*, 60, 40–49. Available from: https://doi.org/10.1016/s0090-4295(02)01683-7

Hollis, A.R. (2020) Strontium plesiotherapy for the treatment of sarcoids in the horse. *Equine Veterinary Education*, 32(S11), 7–11. Available from: https://doi.org/10.1111/eve.13038

Hollis, A.R. & Berlato, D. (2018) Initial experience with high dose rate brachytherapy of periorbital sarcoids in the horse. *Equine Veterinary Education*, 30, 444–449. Available from: https://doi.org/10.1111/eve.12782

Hollister, W., Matthew, A.J., Baust, J.G. & Buskirk, R.G.V. (1998) Effects of freezing on cell viability and mechanisms of cell death in a human prostate cell line. *Molecular Urology*, 2, 3–18. Available from: https://cir.nii.ac.jp/crid/1574231875225830656

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (1987) *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*. Lyon: International Agency for Research on Cancer. (IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS, No. Supplement 7.) 5-FLUOROURACIL (Group 3). Available from: https://www.ncbi.nlm.nih.gov/books/NBK533658/

Ireland, J.L., Wylie, C.E., Collins, S.N., Verheyen, K.L.P. & Newton, J.R. (2013) Preventive health care and owner-reported disease prevalence of horses and ponies in Great Britain. *Research in Veterinary Science*, 95(2), 418–424. Available from: https://doi.org/10.1016/j.rvsc.2013.05.007

Jandova, V., Klukowska-Rötzler, J., Dolf, G., Janda, J., Roosje, P., Marti, E. et al. (2012) Whole genome scan identifies several chromosomal regions linked to equine sarcoids. *Schweizer Archiv für Tierheilkunde*, 154(1), 19–25. Available from: https://doi.org/10.1024/0036-7281/a000288

Jindra, C., Hainisch, E.K. & Brandt, S. (2023) Immunotherapy of equine Sarcoids-from early approaches to innovative vaccines. *Vaccines (Basel)*, 11, 769.

Klein, W.R., Bras, G.E., Misdorp, W., Steerenberg, P.A., de Jong, W.H., Tiesjema, R.H. et al. (1986) Equine sarcoid: BCG immunotherapy compared to cryosurgery in a prospective randomised clinical trial. *Cancer Immunology, Immunotherapy*, 21(2), 133–140. Available from: https://doi.org/10.1007/bf00199861

Knottenbelt, D.C. (2005) A suggested clinical classification for the equine sarcoid. *Clinical Techniques in Equine Practice*, 4(4), 278–295. Available from: https://doi.org/10.1053/j.ctep.2005.10.008

Knottenbelt, D.C. (2019) The equine sarcoid why are there so many treatment options? *Veterinary Clinics of North America: Equine Practice*, 35(2), 243–262. Available from: https://doi.org/10.1016/j.cveq.2019.03.006

Knottenbelt, D.C. & Kelly, D.F. (2000) The diagnosis and treatment of periorbital sarcoid in the horse: 445 cases from 1974 to 1999. *Veterinary Ophthalmology*, 3(2–3), 169–191. Available from: https://doi.org/10.1046/j.1463-5224.2000.00119.x

Knottenbelt, D.C., Patterson-Kane, J.C. & Snalune, K.L. (2015) *Clinical equine oncology*, 1st edition. Amsterdam, The Netherlands: Elsevier.

Knottenbelt, D.C., Watson, A.H., Hotchkiss, J.W., Chopra, S. & Higgins, A.J. (2020) A pilot study on the use of ultra-deformable liposomes containing bleomycin in the treatment of equine sarcoid. *Equine Veterinary Education*, 32(5), 258–263. Available from: https://doi.org/10.1111/eve.12950

Lane, J.G. (1977) The treatment of equine sarcoids by cryosurgery. *Equine Veterinary Journal*, 9(3), 127–133. Available from: https://doi.org/10.1111/j.2042-3306.1977.tb04003.x

Lazary, S., Marti, E., Szalai, G., Gaillard, C. & Gerber, H. (1994) Studies on the frequency and associations of equine leucocyte antigens in sarcoid and summer dermatitis. *Animal Genetics*, 25(Suppl 1), 75–80. Available from: https://doi.org/10.1111/j.1365-2052.1994.tb00406.x

Longley, D.B., Harkin, D.P. & Johnston, P.G. (2003) 5-fluorouracil: mechanisms of action and clinical strategies. *Nature Reviews Cancer*, 3(5), 330–338. Available from: https://doi.org/10.1038/nrc1074

Mair, T.S. & Fews, D. (2016) Detection of sarcoid tissue at 2 cm resection margins following CO_2 laser excision. *Equine Veterinary Journal*, 48(S50), 20. Available from: https://doi.org/10.1111/evj.35_12612

Martens, A., De Moor, A., Demeulemeester, J. & Peelman, L. (2001) Polymerase chain reaction analysis of the surgical margins of equine sarcoids for bovine papilloma virus DNA. *Veterinary Surgery*, 30(5), 460–467. Available from: https://doi.org/10.1053/jvet.2001.25874

Martens, A., De Moor, A., Vlaminck, L., Pille, F. & Steenhaut, M. (2001) Evaluation of excision, cryosurgery and local BCG vaccination for the treatment of equine sarcoids. *Veterinary Record*, 149(22), 665–669. Available from: https://doi.org/10.1136/vr.149.22.665

McCauley, C.T., Hawkins, J.F., Adams, S.B. & Fessler, J.F. (2002) Use of a carbon dioxide laser for surgical management of cutaneous masses in horses: 32 cases (1993–2000). *Journal of the American Veterinary Medical Association*, 220(8), 1192–1197. Available from: https://doi.org/10.2460/javma.2002.220.1192

McConaghy, F.F., Davis, R.E., Reppas, G.P., Rawlinson, R.J., McClintock, S.A., Hutchins, D.R. et al. (1994) Management of equine sarcoids: 1975–93. *New Zealand Veterinary Journal*, 42(5), 180–184. Available from: https://doi.org/10.1080/00480169.1994.35816

Offer, K.S., Dixon, C.E. & Sutton, D.G.M. (2024) Treatment of equine sarcoids: a systematic review. *Equine Veterinary Journal*, 56(1), 12–25. Available from: https://doi.org/10.1111/evj.13935

Offer, K.S., Marchesi, F. & Sutton, D.G.M. (2022) Topical 5-fluorouracil as an adjunct treatment in equine corneolimbal squamous cell carcinoma. *Equine Veterinary Education*, 34(9), e363–e368. Available from: https://doi.org/10.1111/eve.13568

Pucket, J.D. & Gilmour, M.A. (2014) Intralesional 5-fluorouracil (5-FU) for the treatment of eyelid squamous cell carcinoma in 5 horses. *Equine Veterinary Education*, 26(6), 331–335. Available from: https://doi.org/10.1111/j.2042-3292.2011.00259.x

R Core Team. (2022) *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available from: https://www.R-project.org/

Schaffer, P.A., Wobeser, B., Martin, L.E.R., Dennis, M.M. & Duncan, C.G. (2013) Cutaneous neoplastic lesions of equids in the central United States and Canada: 3,351 biopsy specimens from 3,272 equids (2000–2010). *Journal of the American Veterinary Medical Association*, 242(1), 99–104. Available from: https://doi.org/10.2460/javma.242.1.99

Spoormakers, T.J.P., Klein, W.R., Jacobs, J.J.L., Van Den Ingh Th, S.G.A.M., Koten, J.W. & Den Otter, W. (2003) Comparison of the efficacy of local treatment of equine sarcoids with IL-2 or cisplatin/IL-2. *Cancer Immunology, Immunotherapy*, 52(3), 179–184. Available from: https://doi.org/10.1007/s00262-002-0369-0

Stadler, S., Kainzbauer, C., Haralambus, R., Brehm, W., Hainisch, E. & Brandt, S. (2011) Successful treatment of equine sarcoids by topical aciclovir application. *Veterinary Record*, 168(7), 187. Available from: https://doi.org/10.1136/vr.c5430

Stewart, A.A., Rush, B. & Davis, E. (2006) The efficacy of intratumoural 5-fluorouracil for the treatment of equine sarcoids. *Australian Veterinary Journal*, 84(3), 101–106. Available from: https://go.exlibris.link/m9l9v4r3

Tamzali, Y., Borde, L., Rols, M.P., Golzio, M., Lyazrhi, F. & Teissie, J. (2012) Successful treatment of equine sarcoids with cisplatin electrochemotherapy: a retrospective study of 48 cases. *Equine Veterinary Journal*, 44(2), 214–220. Available from: https://doi.org/10.1111/j.2042-3306.2011.00425.x

Tate, L.P. & Tate, K.B. (2019) Equine laser surgery procedures. In: Winkler, C.J. (Ed.) *Laser surgery in veterinary medicine*. Hoboken: John Wiley and Sons, Inc. Available from: https://doi.org/10.1002/9781119486053.ch19

Taylor, S. & Haldorson, G. (2013) A review of equine sarcoid. *Equine Veterinary Education*, 25(4), 210–216. Available from: https://doi.org/10.1111/j.2042-3292.2012.00411.x

Theon, A.P., Galuppo, L.D., Snyder, J.R. & Wilson, W.D. (2006) *Intratumoral administration of cisplatin for treatment of sarcoids in 378 horses*. Paper presented at the Proceedings of the 52nd Annual Convention of the American Association of Equine Practitioners, San Antonio, Texas, USA, 2–6 December, 2006.

Vodenkova, S., Buchler, T., Cervena, K., Veskrnova, V., Vodicka, P. & Vymetalkova, V. (2020) 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. *Pharmacology & Therapeutics*, 206, 107447. Available from: https://doi.org/10.1016/j.pharmthera.2019.107447

SUPPORTING INFORMATION

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