Evaluation of intravitreal topotecan dose levels, toxicity and efficacy for retinoblastoma vitreous seeds: a preclinical and clinical study

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ABSTRACT

Background Current melphalan-based intravitreal regimens for retinoblastoma (RB) vitreous seeds cause retinal toxicity. We assessed the efficacy and toxicity of topotecan monotherapy compared with melphalan in our rabbit model and patient cohort.

Methods Rabbit experiments: empiric pharmacokinetics were determined following topotecan injection. For topotecan (15 μg or 30 μg), melphalan (12.5 μg) or saline, toxicity was evaluated by serial electroretinography (ERG) and histopathology, and efficacy against vitreous seed xenografts was measured by tumour cell reduction and apoptosis induction. Patients: retrospective cohort study of 235 patients receiving 990 intravitreal injections of topotecan or melphalan.

Results Intravitreal topotecan 30 μg (equals 60 μg in humans) achieved the IC50 across the rabbit vitreous. Three weekly topotecan injections (either 15 μg or 30 μg) caused no retinal toxicity in rabbits, whereas melphalan 12.5 μg (equals 25 μg in humans) reduced ERG amplitudes 42%–79%. Intravitreal topotecan 15 μg was equally effective to melphalan to treat WERI-Rb1 cell xenografts in rabbits (96% reduction for topotecan vs saline (p=0.004), 88% reduction for melphalan vs saline (p=0.004), topotecan vs melphalan, p=0.15). In our clinical study, patients received 881 monotherapy injections (48 topotecan, 833 melphalan). Patients receiving 20 μg or 30 μg topotecan demonstrated no significant ERG reductions; melphalan caused ERG reductions of 7.6 μV for every injection of 25 μg (p=0.03) or 30 μg (p<0.001). Most patients treated with intravitreal topotecan also received intravitreal melphalan at some point during their treatment course. Among those eyes treated exclusively with topotecan monotherapy, all eyes were salvaged.

Conclusions Taken together, these experiments suggest that intravitreal topotecan monotherapy for the treatment of RB vitreous seeds is non-toxic and effective.

INTRODUCTION

Vitreous seeds have historically been the most difficult-to-treat aspect of intraocular retinoblastoma (RB).1,2 RB tumours with vitreous seeds are those least likely to be salvaged with radiation3 or intravenous chemotherapy.1,2,4,5 Newer approaches to delivering chemotherapy, including intra-arterial chemotherapy (IAC)6 and direct intravitreal injection of chemotherapy,7,8 have partially overcome the treatment-resistant nature of vitreous seeds and have improved globe salvage rates for RB.9,10 However, the primary chemotherapeutic agent used in both IAC and intravitreal chemotherapy is melphalan, which has been associated with retinal toxicity.8,11,12 Thus, while intravitreal melphalan may be effective, retinal functional loss is common.13,14 Furthermore, the toxicity is dose-dependent15 and worsens with each subsequent melphalan injection delivered.12,13,16

Recently, topotecan has been explored as an alternative chemotherapy agent, both by intravenous,17 intra-arterial18 and intravitreal19,20 routes. Preliminary laboratory and clinical evidence suggests that topotecan may be less toxic than current standard-of-care melphalan.20,21 However, it is unclear if a non-toxic dose of topotecan is clinically effective.21 Further, it is unclear just how effective topotecan is as monotherapy, as many centres have generally used it in combination with melphalan, or have been quick to re-add melphalan back into the regimen if topotecan monotherapy appeared to not achieve adequate tumour control.20 Likewise, the optimal dose of topotecan that best balances efficacy with toxicity as intravitreal monotherapy has not been established.

We recently developed a rabbit xenograft model of RB with vitreous seeds and retinal tumours, which we have used to study the toxicity of IAC, exploring various different IAC drugs.21,24 We have also previously described a complete platform to assess functional and structural retinal toxicity associated with local delivery of various chemotherapeutic agents.11 Here, we use this rabbit model11 and this toxicity evaluation platform,11 to determine the dose of intravitreal topotecan which is effective and non-toxic when delivered as monotherapy. We then corroborate this evidence of non-toxicity with our clinical experience treating RB patients with vitreous seeds with intravitreal topotecan.

METHODS

Statement of research ethics
All animal experiments adhered to the Association for Research in Vision and Ophthalmology
Intravitreal topotecan pharmacokinetics
New Zealand white rabbits (2.8–3.0 kg) were used for all studies. For pharmacokinetic experiments, a 20-gauge valved vitrectomy cannula was inserted 2–3 mm behind the limbus. One microgram topotecan hydrochloride was injected on the opposite side 2–3 mm behind the limbus into the vitreous cavity. Serial vitreous taps were performed through the valved cannula at 30 min, 1 hour, 2 hours, 4 hours and 6 hours. Use of a valved cannula—maintained eye stability and prevented efflux of vitreous contents during manipulations.25 Vitreous samples were immediately placed on dry ice and then stored at −80°C until drug levels were measured.

Vitreous samples were thawed, an internal carbamazepine standard was added and samples were diluted with blank plasma, then deproteinated with acetonitrile. Samples were analysed on a Thermo Scientific TSQ Quantum Ultra mass spectrometer interfaced to a Waters Acquity UPLC system, using methodology we have reported previously.25

Topotecan concentrations were averaged across rabbits at each time point. The resulting mean time-concentration data from each matrix were analysed via non-compartmental analysis (Phoenix WinNonlin V6.4, Pharsight/Certara USA, Princeton, New Jersey, USA) to determine pharmacokinetic parameters, including half-life.

In vitro determination of dosing
Human WERI-Rb1 RB cells (5×10^3) were plated in 96-well plates in the presence of various concentrations of topotecan for 16 hours (five half-lives as determined through the above pharmacokinetic experiments). Topotecan-containing media was then removed, and fresh media added. After 7 days, the Cell-Titer Blue assay (Promega, Madison, Wisconsin, USA) was used to count live cells. Survival curves were graphed with GraphPad, and the IC₉₀ was calculated.

Using the pharmacokinetic parameters determined above, we calculated23 the dose of topotecan that would need to be injected into the eye to achieve the IC₉₀ in the vitreous on the opposite side of the eye for a duration of five half-lives.

Assessment of efficacy of intravitreal topotecan for vitreous seeds in rabbits
Figure 1A depicts our experimental design. RB vitreous seeds were created by intravitreal injection of 1 000 000 WERI-Rb1 cells in 100 µL saline in both eyes of cyclosporine-immunosuppressed rabbits, as we have described previously.23 25 After 2 weeks of growth, the right eyes received three weekly injections of either 15 µg/100 µL topotecan or 12.5 µg/100 µL melphalan, while all left eyes received 100 µL saline.

Two weeks after the final injection, all rabbits were euthanised, and the eyes were removed. For five rabbits, the vitreous of each eye was harvested and digested in 0.5 mg/mL hyaluronidase and 1 mg/mL collagenase overnight at 37°C. Live cells were counted by direct microscopy using trypan blue stain. In four additional rabbits from each treatment group, the entire eyes were submitted for histopathology (two rabbits after receiving three injections and two rabbits after receiving a single injection).

Assessment of ocular toxicity of intravitreal topotecan in rabbits
Four cohorts (n=4–6 rabbits/cohorts) received either topotecan 30 µg (the calculated IC₉₀), topotecan 15 µg (half the calculated IC₉₀), saline (control) or melphalan 12.5 µg (current standard of care).26 In all rabbits within a given cohort, the right eyes received three injections, one injection per week, of the same drug/dose. Figure 1B depicts our experimental design. Electroretinography (ERG; OcuScience, Henderson, Nevada, USA) was performed according to the modified International Standard for Clinical Electrophysiology of Vision protocol for rabbits.27 Intravitreal injections were performed weekly, and always within 1 day following testing. After euthanasia, eyes were harvested and fixed in Davidson’s solution.

Toxicity was defined for every ERG parameter, using our previously published definition.11 22 Briefly, toxicity was deemed significant for a given dose in a rabbit group if there was a 25% reduction in average ERG amplitude, or a 25% prolongation of average implicit time comparing the post-treatment parameter values after three injections with the pretreatment values, if the difference was statistically significant.

Ocular toxicity and efficacy of intravitreal topotecan versus melphalan in patients
Medical records of all patients treated with intravitreal injections at Memorial Sloan-Kettering Cancer Center and Vanderbilt University Medical Center were reviewed. Patients receiving intravitreal topotecan were identified. A second cohort of all patients receiving melphalan as monotherapy were included as a comparator group. Injection number, drug and dose were recorded. ERGs were performed using a previously published protocol for Clinical Electrophysiology of Vision protocol for rabbits.27 Intravitreal injections were performed weekly, and always within 1 day following testing. After euthanasia, eyes were harvested and fixed in Davidson’s solution.

Toxicity was defined for every ERG parameter, using our previously published definition.11 22 Briefly, toxicity was deemed significant for a given dose in a rabbit group if there was a 25% reduction in average ERG amplitude, or a 25% prolongation of average implicit time comparing the post-treatment parameter values after three injections with the pretreatment values, if the difference was statistically significant.
Statistical analyses of rabbit and human efficacy and ERG data

For univariate analysis to compare toxicity in patients, the Wilcoxon signed-rank test was used. For multivariable analysis, to evaluate the toxicity of each drug at different dosages, a linear mixed-effects model was fitted with treatment groups and the repeated measurements (pre or post) for each parameter and each test. Using model-based (least-square) means, the average adjusted change from pretreatment versus post-treatment and the difference in change between different treatment groups (difference-of-differences) were estimated and compared with the Wald test. Our predefined definition of toxicity (see earlier) was used in the rabbit analyses. Data were transformed to better meet normality assumptions and adjusted for heteroscedasticity when necessary. To account for multiple comparisons, Bonferroni-adjusted p values were reported (two-tailed), with adjusted p values less than 0.05 considered statistically significant. The analyses were performed using R V.3.6.3 including packages ‘nlme’ and ‘emmeans’. For experiments in rabbits, ‘pre’ and ‘post’ were defined before and after the three injections. For human experiments, because of the variability between dosing packages, ‘pre’ and ‘post’ were defined on a ‘per patient and intra-eye’ basis without minimum reduction limits, and inter-eye/ intra-patient and intra-eye correlations were taken into account in our modelling.

For efficacy experiments in rabbits comparing paired right eyes receiving topotecan (or melphalan) and contralateral left eyes receiving saline, the paired t-test was used. Relative reduction of cell counts was analysed to compare the difference between two independent groups (topotecan and melphalan rabbit cohorts) using Welch two sample t-test.

RESULTS

In vivo topotecan pharmacokinetics and in vitro determination of expected effective in vivo dose

Peak concentration at the opposite side of the eye was achieved at 2 hours postinjection (figure 2A). The average Cmax at the opposite side of the eye was 0.31 μmol/L. However, the theoretical Cmax (calculated as 1 μg/1.4 mL rabbit vitreous volume) is 1.56 μmol/L. Therefore, compared with the theoretical Cmax, the actual empiric Cmax at the opposite side of the eye achieved 2 hours after injection was ~20% of expected, likely due to rapid efflux during this period of slow diffusion of topotecan across the vitreous. The half-life of topotecan in the rabbit eye was 3.27 hours. We therefore exposed WERI-Rb1 human RB cells to various doses of topotecan for five vitreous half-lives (~16 hours total) and measured live cells 7 days later. The IC50 was 300 nM (figure 2B). We calculated that we would need to inject 30 μg topotecan to sustain this IC50 concentration for 16 hours (five half-lives) at the opposite side of the rabbit eye.

Relative efficacy of intravitreal topotecan versus melphalan to treat RB vitreous seeds in vivo in rabbits

Three weekly injections of 15 μg topotecan killed 96% of vitreous seed tumour cells, compared with saline-treated contralateral eyes (p=0.004, figure 3). Three weekly injections of 12.5 μg melphalan (corresponding to the clinically used dose of 25 μg in patients) killed 88% of cells, compared with saline-treated contralateral eyes (p=0.004; topotecan vs melphalan: p=0.15; figure 3). For additional rabbits in each cohort, the entire eyes were harvested and submitted for histopathology. Residual RB cells in the topotecan-treated eyes were TUNEL positive, suggesting that the ~4% of ‘remaining’ cells counted in the vitreous seed quantitation assay were likely in the process of dying as well.

Toxicity of various doses of intravitreal topotecan compared with melphalan in rabbits

While there was no worsening of ERG parameters in the saline control group, melphalan caused significant worsening of almost all ERG parameters, with reductions in ERG amplitudes between 42% and 79% (figure 4A). These ERG changes...
Laboratory science

occurred in every rabbit within the cohort, with a median of 9 (IQR: 7–9, out of 18) parameters affected per rabbit. Similarly, implicit times were prolonged. Histopathology demonstrated severe atrophy affecting all retinal layers, worst near the injection sites (figure 4B–D).

In contrast, rabbits in the 15 µg and 30 µg topotecan cohorts did not experience any statistically or clinically meaningful worsening of ERG parameters (figure 4A). Even at twice the clinically effective dose, multiple repeated weekly intravitreal topotecan injections did not cause retinal toxicity. No other signs of toxicity were observed on clinical examination, and histopathology showed none of the retinal damage that was seen in the melphalan-treated groups, with retinas of eyes treated with topotecan being histologically indistinguishable from saline-treated control eyes (figure 4B–F).

Comparative toxicity in patients receiving intravitreal topotecan at various doses compared with melphalan

In 41 patients, 108 intravitreal injections of topotecan were given to 42 eyes. Of these 108 injections, 48 injections consisted of topotecan as intravitreal monotherapy, at dosages of either 30 µg (18 injections), 20 µg (29 injections) or 10 µg (one injection). In general, the lower dose of 20 µg was used until ~2017, and 30 µg was used beginning in mid-2017, when it was felt that the efficacy-versus-toxicity balance warranted an increase in dose (the single treatment with 10 µg was given in 2014). Preinjection and postinjection ERG data were available for 384 injections. Injections were excluded if they were still receiving concomitant IAC, if pretreatment ERGs were undetectable (<5 μV) were excluded. Six additional injections were excluded because they were still receiving concomitant IAC. Ultimately, this left 25 topotecan monotherapy injections (from 14 eyes) with evaluable ERGs for analysis (11 at 20 µg and 14 at 30 µg).

In the comparator group, 882 intravitreal melphalan injections were given to 210 eyes of 194 patients. Of these, 833 injections (205 eyes) consisted of melphalan as intravitreal monotherapy (99 injections at 25 µg, 732 injections at 30 µg, 2 injections at 40 µg). Preinjection and postinjection ERG data using the previously described and validated abbreviated clinical protocol were available for 384 injections. Injections were excluded if they were still receiving concomitant IAC, if pretreatment ERGs were undetectable (<5 μV) were excluded.
Figure 4  Absence of retinal toxicity with various doses of intravitreal topotecan, compared with melphalan. (A) Retinal function. Electroretinography was performed weekly, 1 day prior to each of the three planned injections, as well as 1 week after the final injection (immediately prior to euthanising the rabbit). Retinal responses to scotopic 100 mcd flashes, scotopic 3000 mcd flashes, scotopic 10 000 mcd flashes, photopic 3000 mcd flashes and 30 Hz flicker flashes were recorded. A-wave and B-wave amplitudes, and A-wave and B-wave implicit times were recorded (except for the 30 Hz flicker, for which there is only a B wave). Shaded areas on the graphs represent 95% CIs. No toxicity (see the Methods section for toxicity criteria) was observed for any parameter in the saline-treated control eyes, as well as in the cohorts treated with either 15 µg or 30 µg of topotecan. However, significant toxicity was seen in the cohort of rabbits treated with 12.5 µg of melphalan. Graphs of amplitudes are shown, but similar results were seen for implicit times, as well. For those particular tests where significant toxicity was seen, per cent change and p values for estimates of trend are shown alongside the particular graph. P values of the difference between groups are shown at the top of each graph. (B–F) Histopathology of treated eyes demonstrating (B) normal retinal architecture in untreated eyes and in (C) the saline-treated eyes. (D) In contrast, eyes treated with 12.5 µg melphalan showed significant retinal atrophy on histopathology (arrow shows the location of loss of outer retinal architecture). Eyes treated with intravitreal injections of (E) 15 µg topotecan, or (F) 30 µg topotecan were histologically indistinguishable from saline-treated (or untreated) eyes. Retinal detachments are artefactual. NS=not significant.
were ‘undetectable’ (<5 μV), or if multiple injections occurred between the preinjection and postinjection ERGs. Thus, the final group included for analysis of toxicity and ERGs included 225 intravitreal melphalan monotherapy injections (66 at 25 μg, 159 at 30 μg).

In a univariate analysis, patients receiving melphalan experienced a 7.29 μV reduction in ERG amplitude, per injection (p<0.001), with no significant difference in the amount of reduction between those receiving 25 μg or 30 μg of melphalan. In contrast, patients receiving topotecan experienced no reduction in ERG amplitude, in either the 20 μg subcohort or the 30 μg subcohort (figure 5).

In a mixed effect model, patients receiving melphalan experienced a 7.55 μV reduction in ERG amplitude, per injection (p<0.001), consisting of 7.58 μV reduction per 25 μg injection (p=0.03), and 7.37 μV reduction per 30 μg injection (p<0.001). In contrast, in the mixed effect models, patients receiving topotecan at either 20 μg or 30 μg experienced no reduction in ERG amplitude (figure 5).

### Efficacy of intravitreal topotecan compared with melphalan in patients

There were 23 patients (23 eyes) who were treated with intravitreal topotecan for whom a complete clinical course was available for review (follow-up: 23.5±18.8 months). Of these six, five were treated with 30 μg (all after October 2017), and one was treated with 20 μg. The comparator group consisted of 66 patients (70 eyes) whose entire intravitreal treatment course consisted solely of melphalan monotherapy, receiving a mean of 4.0±2.4 injections (follow-up: 30.9±26.0 months). In this melphalan monotherapy group, seed eradication and globe salvage was achieved in 65/70 (92.9%) of eyes. It is difficult to evaluate the true efficacy of topotecan in this cohort as the majority of patients who received intravitreal topotecan also received intravitreal melphalan at some point during the course of treatment, and so we cannot definitively attribute the seed eradication to the topotecan in those cases. Only six patients received topotecan monotherapy exclusively throughout their intravitreal treatment course, and while the vitreous seeds were successfully eradicated in all of these patients (having received a mean of 1.8±0.75 injections), it is possible that there might have been selection bias whereby the patients with the least significant vitreous tumour burden were most likely to receive only topotecan. Future randomised studies are needed to evaluate the relative efficacy of topotecan versus melphalan.

### DISCUSSION

To assess the efficacy, toxicity and optimal therapeutic dose of intravitreal topotecan monotherapy for vitreous seeds, we performed several in vitro experiments, in vivo experiments in our rabbit model, and we report our clinical experience using intravitreal topotecan in RB patients. Our pharmacokinetic experiments and in vitro experiments calculated an optimal dose range of 15–30 μg in rabbits (equivalent to 30–60 μg in the larger human eye). Our in vivo efficacy experiments in our rabbit xenograft model demonstrate that 15 μg topotecan is highly effective at eradicating vitreous seeds, with efficacy equivalent to standard dose melphalan. Our in vivo toxicity experiments in rabbits demonstrate that multiple injections of topotecan, even up to 30 μg, do not cause retinal functional or structural toxicity, in contrast to melphalan. Finally, in the clinical study, our experience with intravitreal topotecan monotherapy confirms that 30 μg in humans (equivalent to 15 μg in the smaller rabbit eye) does not cause retinal toxicity in patients.

Evidence from animal models and clinical experience suggests that currently used melphalan may be associated with retinal toxicity. Topotecan has been proposed as an alternative agent with efficacy against RB, and it has been incorporated into chemotherapy regimens by intravenous, intra-arterial and recently intravitreal routes. However, topotecan has always been used in combination with other drugs for intravenous and intra-arterial regimens. When topotecan was initially explored for intravitreal use, it was likewise combined with melphalan in an effort to increase its toxicity in calcitrant eyes, but Nadelmann et al have shown that combining topotecan with melphalan still causes the expected toxicity from melphalan. Recently, single-agent topotecan has been proposed for vitreous seeds. While some have reported good results, there is much variability in the doses used and many reports still ultimately include melphalan in combination, presumably because of a perceived lack of adequate efficacy at the doses selected for topotecan. A previous evaluation of the toxicity of intravitreal topotecan in an animal model selected a 5 μg dose (equivalent to 10 μg in humans), far less than the doses currently used in clinical practice, six-times less than the dose that we calculate to achieve the IC$_{90}$ and three-times less than the dose we demonstrate to be clinically effective. Importantly, we demonstrate no toxicity with 15 μg or even 30 μg of intravitreal topotecan.

We took an evidence-based approach, rather than an exploratory trial-and-error approach, to determine the ideal dose of topotecan to study. Since each individual injection can only be given at a single location within the globe, while seeds are often diffuse throughout the vitreous cavity, the goal was to identify the concentration required at the farthest-most side of the vitreous to eradicate vitreous seeds at this farthest location. There are different factors to consider, including the rate of diffusion and the rate of efflux. The amount of drug present at the opposite side of the eye at various time-points following injection was therefore determined empirically. In vitro cytotoxicity experiments were then performed to determine the minimum concentration necessary at that location based on the pharmacokinetic parameters found in vivo, and we then calculated the initial dose that would have been required to be injected to achieve the desired concentration for a sustained length of time (five half-lives) at that farthest point. This systematic approach to dose-finding is superior to selecting several doses in a more random fashion. Since this approach identifies the required concentration at the end of five-half-lives, and at the farthest location in the eye with the lowest exposure levels, this likely represents a high-end estimate—most of the vitreous cavity is exposed to higher concentrations, and indeed at earlier time points the concentration is higher at all locations than it is at the end of the fifth half-life. In addition, this calculated effective dose assumes a single injection, whereas in practice, one would always give multiple injections. Therefore, we also explored half the calculated dose (15 μg instead of the full 30 μg). Since 15 μg was shown in our rabbit model to be as effective as current melphalan doses, we then explored toxicity at the full 30 μg in our rabbit model as well, and we demonstrate that there is a wide therapeutic window with topotecan.

Similarly, the ERG data of topotecan-treated patients corroborated our rabbit findings that doses of 20 μg or even 30 μg did not cause retinal toxicity or ERG reductions. In contrast, in our mixed effect model, melphalan caused a per-injection reduction in retinal function equivalent to 7.55 μV for every
Laboratory science

Figure 5  Changes in retinal function in topotecan-treated versus melphalan-treated eyes of patients with retinoblastoma vitreous seeds. Within each cohort or subcohort (delineated within a box), the top panel represents the univariate analysis, with each ‘string’ representing the electroretinography (ERG) changes with a single intravitreal injection. The bottom panel within each pair represents the results of the mixed-effect modelling, accounting for inter-eye/intra-patient and intra-eye correlations, with the appropriate statistical analysis results labelled on the panel.

(A–B) ERG amplitude changes per injection for patients treated with topotecan (A), or melphalan (B). Topotecan caused no significant reduction in ERG parameters, whereas significant reductions in ERG amplitudes were seen with each injection of melphalan (7.55 μV per injection, p<0.001). (C–F) ERG amplitude changes by drug and dose. (C–D) represent subcohorts of the full topotecan-treated cohort presented in (A), and (E–F) represent subcohorts of the full melphalan-treated cohort presented in (B). Topotecan caused no reduction in ERG parameters at either 20 μg (C) or 30 μg (D), whereas significant reductions in ERG amplitudes were seen with each injection of either 25 μg melphalan (E; 7.58 μV per injection, p=0.03) or 30 μg melphalan (F; 7.57 μV per injection, p<0.001). NS=not significant.

meltphalan injection, consistent with previous publications by our group. There are two commonly used formulations of melphalan: (traditional) melphalan hydrochloride and captisol-stabilised propylene glycol-free melphalan. In the rabbit experiments, all rabbits were treated with traditional melphalan hydrochloride. In the patient cohort, patients treated up until 2015 (at VUMC) and up until 2016 (at MSKCC) were treated with melphalan hydrochloride, while all patients treated after those dates were treated with the newer propylene glycol-free formulation. We have previously shown that the efficacy and the
toxicity of both formulations do not differ. However, it should be pointed out that not all eyes receiving melphalan will necessarily experience worsened visual function. As seen in figure 5, while there was a reduction in ERG amplitudes on average, some eyes experienced little or no ERG reductions. It should also be pointed out that macular toxicity (including cystoid macular oedema), which has been reported to occur occasionally with intravitreal injections, might not result in measurable reduction in retina-wide, full-field ERG. The specific factors influencing retinal toxicity in a given patient are not clear.

CONCLUSIONS
Taken together, our preclinical and clinical findings support that topotecan 30 μg (equivalent to 15 μg in our rabbit models) appears to cause no retinal toxicity in the rabbit model or in patients. Our rabbit model data indicate that topotecan might be equally effective to melphalan, supporting the need for future clinical studies that directly compare the efficacy in patients.

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Data are available upon reasonable request. For laboratory data, contact Anthony.B.daniels@vumc.org. For deidentified patient data, contact Anthony.B.daniels@vumc.org and abramod@mskcc.org.

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