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 IC₉₀ 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) 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 11–13 Thus, while intravitreal melphalan may be effective, retinal functional loss is common.12 14 Furthermore, the toxicity is dose-dependent and worsens with each subsequent melphalan injection delivered.12 13 16

Recently, topotecan has been explored as an alternative chemotherapeutic agent, both by intravenous,19 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,22 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.23 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 model 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
Statement on Animal Use and were performed under the auspices of the Vanderbilt Institutional Animal Care and Use Committee.

**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. 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 deproteinised 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. 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 (5×10⁴) 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 calculated 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. 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/cohort) 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). 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. Electrotetroretinography (ERG; OcuScience, Henderson, Nevada, USA) was performed according to the modified International Standard for Clinical Electrophysiology of Vision protocol for rabbits. 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. 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,
and validated, abbreviated ERG protocol. For efficacy, we included all treated patients for which complete records were available. Our toxicity analyses included only those monotherapy injections for which this ERG protocol was performed prior to the intravitreal injection, as well as subsequent to the injection, and patients who also received concomitant IAC between the two ERGs were excluded. Ocular and systemic adverse events, as well as clinical outcomes, were recorded.

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 ‘nlim’ 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 ‘nlme’ and ‘emmeans’. The analyses were performed using R V.3.6.3 including packages ‘nlme’ and ‘emmeans’. 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 animal experiments, ‘pre’ and ‘post’ were defined before and after the three injections. For human experiments, the Wilcoxon signed-rank test was used for univariate analysis to compare toxicity in patients, the Wilcoxon signed-rank test was used. For multivariable analysis, the Wilcoxon signed-rank test was used.
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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 showed ‘undetectable’ (<5 μV) ERG (9 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 showed ‘undetectable’ (<5 μV) ERG (9 injections at 30 μg, 2 injections at 40 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 showed ‘undetectable’ (<5 μV) ERG (9 injections at 30 μg, 2 injections at 40 μg).
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 7.57 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 studies 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 efficacy in recalcitrant 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 IC₉₀ 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
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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.

melphalan injection, consistent with previous publications by our group.12 37 38 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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CONFLICTS OF INTEREST

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REFERENCES

1 Shields CL, Mashayekhi A, Au AK, et al. The International classification of retinoblastoma predicts chemoreduction success. Ophthalmology 2006;113:2376–80.
2 Shields CL, Honavar SG, Meadows AT, et al. Chemoreduction plus focal therapy for retinoblastoma: factors predictive of need for treatment with external beam radiotherapy or enucleation. Am J Ophthalmol 2002;133:657–64.
3 Abramson DH, Beaverson KL, Chang ST, et al. Outcome following initial external beam radiotherapy in patients with Reese-Ellsworth group Vb retinoblastoma. Arch Ophthalmol 2004;122:1316–21.
4 Murphy AE, Villablancia JG, Deegan WE, et al. Chemotherapy plus local treatment in the management of intraretinal retinoblastoma. Arch Ophthalmol 1996;114:1348–56.
5 Daniels AB, Patel SN, Milam RW, et al. Effect of intravitreal chemotherapy regimen on globe salvage success rates for retinoblastoma based on disease Class—A meta-analysis. Cancers 2021;13:2216.
6 Abramson DH, Dunkel IJ, Brodie SE, et al. A phase VII study of direct intraaerial (ophthalmic artery) chemotherapy with melphalan for intraretinal retinoblastoma initial results. Ophthalmology 2008;115:1404 e1391:1398–404.
7 Munier FL, Soliman S, Moulin AP, et al. Profiling safety of intravitreal injections for retinoblastoma using an anti-reflux procedure and sterilisation of the needle track. Br J Ophthalmol 2012;96:1084–7.
8 Munier FL, Gaillard M-C, Balmer A, et al. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: from prohibition to conditional indications. Br J Ophthalmol 2012;96:1078–83.
9 Abramson DH, Daniels AB, Marr BP, et al. Intra-Arterial chemotherapy (ophthalmic artery Chemosurgery) for group D retinoblastoma. PLoS One 2016;11:e0146582.
10 Berry JL, Shah S, Bechtold M, et al. Long-term outcomes of group D retinoblastoma eyes during the intravitreal melphalan era. Pediatr Blood Cancer 2017;64. doi:10.1002/pbc.26696. [Epub ahead of print: 24 06 2017].
11 Daniels AB, Froehler MT, Kim AH, et al. Rabbit model of intra-arterial chemotherapy toxicity demonstrates retinopathy and vasculopathy related to drug and dose, not procedure or approach. Invest Ophthalmol Vis Sci 2019;60:954–64.
12 Francis JH, Schaiquech F, Buitrago E, et al. Local and systemic toxicity of intravitreal melphalan for vitreous seeding in retinoblastoma: a preclinical and clinical study. Ophthalmology 2014;121:1810–7.
13 Xue K, Ren H, Meng F, et al. Ocular toxicity of intravitreal melphalan for retinoblastoma in Chinese patients. BMC Ophthalmol 2019;19:61.
14 Hishe J, Liao A, Francis JH, et al. Comparison of efficacy and toxicity of intravitreal melphalan formulations for retinoblastoma. PLoS One 2020;15:e0235016.
15 Liao A, Hishe J, Francis JH, et al. Toxicity and efficacy of intravitreal melphalan for retinoblastoma: 25 μg versus 30 μg. Retina 2021;41:208–12.
16 Smith SJ, Smith BD, Moroney BG. Ocular side effects following intravitreal injection therapy for retinoblastoma: a systematic review. Br J Ophthalmol 2014;98:292–7.
17 Qaddoumi I, Billups CA, Tagen M, et al. Topotecan and vincristine combination is effective against advanced bilateral intraretinal retinoblastoma and has manageable toxicity. Cancer 2012;118:5663–70.
18 Man BP, Brodie SE, Dunkel IJ, et al. Three-Drug intra-arterial chemotherapy using simultaneous carboplatin, topotecan and melphalan for intraretinal retinoblastoma: preliminary results. Br J Ophthalmol 2012;96:1300–3.
19 Rao R, Honavar SG, Sharma V, et al. Intravitreal topotecan in the management of refractory and recurrent vitreous seeds in retinoblastoma. Br J Ophthalmol 2018;102:490–5.
20 Nadelmann J, Francis JH, Brodie SE, et al. Is intravitreal topotecan toxic to retinal function? Br J Ophthalmol 2021;105:1016–8.
21 Buitrago E, Del Sole MJ, Toribondi A, et al. Ocular and systemic toxicity of intravitreal topotecan in rabbits for potential treatment of retinoblastoma. Exp Eye Res 2015;138:103–9.
22 Ghassemi S, Shields CL, Ghadimi H, et al. Combined intravitreal melphalan and topotecan for refractory or recurrent vitreous seeding from retinoblastoma. JAMA Ophthalmol 2014;132:936–41.
Laboratory science

23 Daniels AB, Froehler MT, Pierce JM, et al. Pharmacokinetics, tissue localization, toxicity, and treatment efficacy in the first small animal (rabbit) model of intra-arterial chemotherapy for retinoblastoma. *Invest Ophthalmol Vis Sci* 2018;59:446–54.

24 Oatess TL, Chen PH, Daniels AB, et al. Severe Periocular Edema after Intraarterial Carboplatin Chemotherapy for Retinoblastoma in a Rabbit (*Oryctolagus cuniculus*) Model. *Comp Med* 2020;70:176–82.

25 Daniels AB, Pierce JM, Chen S-C. Complete preclinical platform for intravitreal chemotherapy drug discovery for retinoblastoma: assessment of pharmacokinetics, toxicity and efficacy using a rabbit model. *MethodsX* 2021;113:101358.

26 Berry JL, Bechtold M, Shah S, et al. Not All Seeds Are Created Equal: Seed Classification Is Predictive of Outcomes in Retinoblastoma. *Ophthalmology* 2017;124:1817–25.

27 Gjörloff K, Andréasson S, Ehinger B. Standardized full-field electroretinography in rabbits. *Doc Ophthalmol* 2004;109:163–8.

28 Liu CY, Jonna G, Francis JH, et al. Non-selectivity of ERG reductions in eyes treated for retinoblastoma. *Doc Ophthalmol* 2014;128:13–23.

29 Brodie SE, Munier FL, Francis JH, et al. Persistence of retinal function after intravitreal melphalan injection for retinoblastoma. *Doc Ophthalmol* 2013;126:79–84.

30 Brodie SE, Pierre Gobin Y, Dunkel IJ, et al. Persistence of retinal function after selective ophthalmic artery chemotherapy infusion for retinoblastoma. *Doc Ophthalmol* 2009;119:13–22.

31 Tse BC, Steinle JJ, Johnson D, et al. Superselective intraophthalmic artery chemotherapy in a nonhuman primate model: histopathologic findings. *JAMA Ophthalmol* 2013;131:903–11.

32 Steinle JJ, Zhang Q, Thompson KE, et al. Intra-ophthalmic artery chemotherapy triggers vascular toxicity through endothelial cell inflammation and leukostasis. *Invest Ophthalmol Vis Sci* 2012;53:2439–45.

33 Ghassemi F, Amoli FA. Pathological findings in enucleated eyes after intravitreal melphalan injection. *Int Ophthalmol* 2014;34:533–40.

34 Aziz HA, Kim JW, Munier FL, et al. Acute hemorrhagic retinopathy following intravitreal melphalan injection for retinoblastoma: a report of two cases and technical modifications to enhance the prevention of retinal toxicity. *Ocul Oncol Pathol* 2017;3:34–40.

35 Brennan RC, Qaddoumi I, Mao S, et al. Ocular salvage and vision preservation using a Topotecan-Based regimen for advanced intraocular retinoblastoma. *JCO* 2017;35:72–7.

36 Francis JH, Gobin YP, Dunkel IJ, et al. Carboplatin +/- topotecan ophthalmic artery chemosurgery for intraocular retinoblastoma. *PLoS One* 2013;8:e72441.

37 Francis JH, Brodie SE, Marr B, et al. Efficacy and toxicity of Intravitreous chemotherapy for retinoblastoma: four-year experience. *Ophthalmology* 2017;124:488–95.

38 Bogan CM, Pierce JM, Doss SD, et al. Intravitreal melphalan hydrochloride vs propylene glycol-free melphalan for retinoblastoma vitreous seeds: efficacy, toxicity and stability in rabbits models and patients. *Exp Eye Res* 2021;204:108439.

39 Panthagani J, Montecinos P, Lopez JP, et al. Cystoid macular edema following intravitreal chemotherapy treatment for retinoblastoma. *Pediatr Blood Cancer* 2020;67:e28348.