Combination of Carboplatin and Bevacizumab Is an Efficient Therapeutic Approach in Retinoblastoma Patient-Derived Xenografts

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PUBLISHED: November 24, 2015
ACCEPTED: July 11, 2016
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Retinoblastoma (Rb) is a rare childhood cancer of the retina with a survival rate of 95% in children living in high-income countries, after appropriate therapies such as chemotherapy, local ophthalmologic treatment, and radiotherapy. However, due to inactivation of the RB1 gene, all bilateral and almost 15% of unilateral retinoblastoma patients have a higher risk of secondary cancers, especially sarcomas. Hence, new nonmutagen treatments are warranted. Therefore, we investigated the efficacy of therapy using anti-VEGF antibody bevacizumab, either alone or with carboplatin, in well-characterized Rb patient-derived xenografts (PDXs).

PURPOSE. Retinoblastoma (Rb) is a rare childhood cancer of the retina with a survival rate of 95% in children living in high-income countries, after appropriate therapies such as chemotherapy, local ophthalmologic treatment, and radiotherapy. However, due to inactivation of the RB1 gene, all bilateral and almost 15% of unilateral retinoblastoma patients have a higher risk of secondary cancers, especially sarcomas. Hence, new nonmutagen treatments are warranted. Therefore, we investigated the efficacy of therapy using anti-VEGF antibody bevacizumab, either alone or with carboplatin, in well-characterized Rb patient-derived xenografts (PDXs).

METHODS. Three Rb PDXs previously established and characterized, RB102, RB111, and RB200, have been treated using carboplatin, bevacizumab, or carboplatin + bevacizumab. In order to define antitumor responses, various quantitative PCR and histopathologic analyses have then been performed on tumors collected at the end of experiments.

RESULTS. In all treated PDX models, we have observed a high and significant improvement of chemotherapy-induced in vivo efficacy by the antiangiogenic antibody. The overall response rate, lower than ~0.5, was 48%, 27%, and 86% after carboplatin, bevacizumab, and carboplatin + bevacizumab, respectively (carboplatin versus carboplatin + bevacizumab; P < 10<sup>-2</sup>; bevacizumab versus carboplatin + bevacizumab; P < 10<sup>-3</sup>). In the RB200 PDX, such a result was also observed when bevacizumab was combined with lower doses of carboplatin. Quantitative PCR and histopathologic analyses have been performed and confirmed the impact of the bevacizumab-based treatments on various angiogenic markers.

CONCLUSIONS. Overall, our in vivo results confirm the interest in antiangiogenic therapy for the treatment of Rb in combination with carboplatin and provide a robust rationale for testing this combination in the clinical setting for Rb patients.

Keywords: bevacizumab, carboplatin, patient-derived xenografts, PDX, retinoblastoma
tival,17 or intravitreal chemotherapy18–21 have been developed, showing a significant reduction in the need for EBRT.5,22,23 However, in bilateral Rb with genetic susceptibility to tumors, chemotherapy can also increase the risk of secondary cancers and is often associated with other toxicities, such as ototoxicity,24 clearly indicating a high need for alternative and less toxic therapies.

In this view, various observations support the concept of angiogenesis inhibition in Rb, as already reported.25 First, Rb tumors are characterized by vascular dilation, frequently associated with voluminous tumors.1 Second, it has been shown that vascular endothelial growth factor (VEGF) was highly expressed in retinoblastoma,26–28 correlated to aggressive features of the disease, such as optic nerve invasion28 and poor patient outcome.27,29–31 In particular, VEGF was found to be highly expressed in residual tumor cells after neoadjuvant chemotherapy and correlated to local invasion and worse prognosis.32 Similarly, it has been observed in overexpression of vegfa, flt-1, kdr, and bfl1-α transcripts and high secretion of VEGF-A in chemotherapy-refractory patients.33 Third, various molecular observations have highlighted the potential role of angiogenesis in Rb: for example, during sustained hypoxia, p53 downregulates VEGF expression through the Rb pathway in a p21-dependent manner34; and heparanase (HPSE), hypoxia-inducible factor (HIF-1α), and VEGF promote malignant progress of retinoblastoma.35 Finally, a number of therapeutic observations favor antiangiogenic therapy in Rb: (1) VEGF-targeted antisense gene therapy has been shown to be efficient in treating retinoblastoma cell line SO-RB50 in both in vitro and in vivo experiments36; (2) bevacizumab inhibits differentiation of retinoblastoma cells through ERK1/2 activation37 and angiogenesis and growth of retinoblastoma38; (3) pigment epithelium-derived factor (PEDF), an angiogenesis inhibitor, inhibits growth of retinoblastoma by antiangiogenic activity39; and (4), intravitreal carboplatin plus bevacizumab induced tumor regressions among 7 of 11 children treated for refractory Rb.40 Overall, these observations support the use of antiangiogenic therapy in Rb patients, especially in combination with chemotherapy.

In order to investigate such a therapeutic approach, we tested therapy using the anti-VEGF antibody bevacizumab alone or with carboplatin, in well-characterized Rb patient-derived xenografts (PDX) developed in the laboratory.41

**MATERIALS AND METHODS**

**RB Patient-Derived Xenografts**

Three previously established and characterized Rb PDXs have been used for in vivo experiments, RB102, RB111, and RB200.41 These three PDXs derive from unilateral, nonfamilial, RB cases.

**Rb1 Molecular Analyses**

Patients’ tumors and xenografts were studied as part of routine diagnostic analyses, using previously published protocols.42,43 Briefly, all RB1 coding sequence and flanking intronic junctions were captured and then sequenced using an array-based platform (Illumina, Santa Clara, CA, USA) in order to identify point mutations and large rearrangements. Microsatellite and methylation analyses were used to searching for loss of heterozygosity (LOH) and promoter methylation, respectively. Two intragenic microsatellite markers (D13S153 and RB14) and three extragenic markers on both centromeric and telomeric parts were used for LOH determination. Tumor DNAs were digested with the methylation-sensitive enzyme Hpa II, using corresponding germline DNAs as controls and following analysis by fluorescent quantitative multiplex PCR.

**Compounds**

Carboplatin (generic provided by Hospira, Lake Forest, IL, USA) was intraperitoneally administered at a dosage between

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**Figure 1.** In vivo responses of Rb PDXs to bevacizumab, carboplatin, and bevacizumab + carboplatin. Rb102 (A), RB111 (B), and RB200 (C) were treated with bevacizumab, 15 mg/kg twice a week (green square), carboplatin, 66 mg/kg once a week (blue triangle), or both (red circle). Controls (white circle) were treated with isotypic antibody, 15 mg/kg twice a week. Tumor growth was evaluated by plotting the mean ± SD of the relative tumor volume per group.
22 mg/kg and 66 mg/kg once a week according to experiments. Bevacizumab (Avastin; Hoffmann-La Roche AG, Basel, Switzerland) was intraperitoneally administered at a dosage of 15 mg/kg once a week for 4 weeks. Mice in the control group were intraperitoneally treated using the control isotypic antibody rituximab (Mabthera; Roche) at a dosage of 15 mg/kg once a week for 4 weeks.

In Vivo Experiments

Swiss nude mice, 4 to 6 weeks old, bred in the animal facilities at Institut Curie, were used for in vivo experiments. Fragments of 30 to 60 mm³ were grafted subcutaneously into the interscapular fat pad. When tumors reached a size of 60 to 200 mm³, 10 mice per group were randomly assigned to the

**FIGURE 2.** Waterfall plots after in vivo treatment with bevacizumab, carboplatin, and bevacizumab + carboplatin. Individual mouse responses are plotted after administration of bevacizumab (A), carboplatin (B), and bevacizumab + carboplatin (C), with an ORR lower than −0.5 or −0.75 of 48% (29%), 27% (5%), and 86% (52%), respectively. (D) Proportion of responding treated mice (y-axis) was notified according to the quality of response (x-axis).

**FIGURE 3.** In vivo responses of the Rb200 PDXs to bevacizumab and carboplatin used at lower dosages. Rb200 was treated by using bevacizumab, 15 mg/kg twice a week (green square), carboplatin, 22 mg/kg (light blue triangle) or 44 mg/kg (dark blue triangle) once a week, or both (bevacizumab + carboplatin, 22 mg/kg [pink circle]; bevacizumab + carboplatin, 44 mg/kg [red circle]). Controls (white circle) were treated with isotypic antibody, 15 mg/kg twice a week. Tumor growth was evaluated by plotting the mean of the relative tumor volume ± SD per group.
control or treatment groups, and treatment was started. Tumor growth was evaluated biweekly by measuring the two perpendicular diameters of tumors with a caliper. Mice that received xenografts (xenografted mice) were euthanized when their tumor reached a volume of 2500 mm³.

Tumor volume \( V \) was calculated according to the following formula: \[ V = a \times b^2/2 \], where \( a \) and \( b \) are the largest and smallest perpendicular tumor diameters, respectively. Relative tumor volumes (RTV) were calculated from the following formula: \[ RTV = \frac{V_x}{V_1} \], where \( V_x \) is the tumor volume on day \( x \) and \( V_1 \) is the tumor volume at initiation of therapy (day 1). Growth curves were obtained by plotting the mean values of RTV on the \( y \)-axis relative to time (\( x \)-axis, expressed as days after start of treatment). Antitumor activity was evaluated according to tumor growth inhibition (TGI), calculated according to the following formula: \[ \text{TGI} = \frac{100}{C_0} \left( \frac{\text{RTV}_t}{\text{RTV}_c} - 1 \right) \], where \( \text{RTV}_t \) is the median RTV of treated mice, and \( \text{RTV}_c \) is the median RTV of controls, both at the time point when the antitumor effect was optimal. The value 50% TGI was considered the limit for a meaningful biological effect. Statistical significance of differences observed between the individual RTVs corresponding to the treated mice and those in the control group were calculated using two-tailed Student's \( t \)-test.

Moreover, to evaluate response to treatments observed in the 3 models according to individual mouse variability, we decided to consider each mouse as one tumor-bearing entity. Hence, in all in vivo experiments, a relative tumor volume variation (RTVV) of each treated mouse was calculated from the following formula: \[ \left( \frac{V_t}{V_c} \right) - 1 \], where \( V_t \) is the volume of the treated mouse and \( V_c \) the median volume of the corresponding control group at a time corresponding to the end of treatment. Then, for each treated mouse, we calculated \( \left( \frac{\text{RTVV}}{C_0} \right) - 1 \). A tumor was considered responding to therapy when \( \left( \frac{\text{RTVV}}{C_0} \right) - 1 \) was lower than \( -0.5 \). A chi-square test was used to perform 2 to 2 treatment comparisons.

The animals were maintained under specified pathogen-free sterile conditions with rodent food and water ad libitum. The experimental protocol and animal housing were in accordance with institutional guidelines set forth by the French Ethical Committee (agreement C75–05-18, France) and the ethics committee of the Institut Curie, which approved this project. All experiments have been performed in adherence to ARVO statement for the use of animals in ophthalmic and vision research.

**RNA Extraction**

Total RNA was isolated from cell sorting fractions by using the RNeasy Plus micro kit (Qiagen, Inc., Hilden, Germany). The RNA quality was assessed by capillary electrophoresis (Bioanalyzer; Agilent, Inc., Santa Clara, CA, USA) based on the RNA 6000 Pico LabChip kit (Agilent) that assigns per sample quantification and integrity.
sequences chosen for the primers and the absence of single nucleotide polymorphisms. To avoid amplification of contaminating genomic DNA, 1 of the 2 primers was placed at the junction between 2 exons. Agarose gel electrophoresis was used to verify the specificity of PCR amplicons. The synthesis of cDNA and PCR analysis were carried out under conditions previously described.44 The expression of 22 genes was studied, of which 7 were determined to be orthologous mouse (Mm) and human (Hs or not indicated) genes that could be classified as (1) angiogenic: HIF1a, VEGFA Hs/Mm, PGF Hs/Mm, VEGFR1 Hs/Mm, and VEGFR2 Hs/Mm; (2) proliferative: MKI67, MYC, and BBC3 (PUMA); (3) apoptotic: BCL2, p21, CDKN1A, and GADD45A; (4) EMT: VIM, and CDH1; (5) NFKB: TNFAIP3; (6) cell cohesive: PECAM1 Hs/Mm and ICAM1; (7) autophagous: CXCR4 Hs/Mm, and CXCL12 Hs/Mm; (8) hormone receptor: ESR1; and (9) carboplatin resistant: FOXM1, CCNE1, and ERCC1.

**Real-time Reverse Transcription PCR**

Quantitative values were obtained from the cycle threshold (Ct) value at which the increase in the fluorescence signal associated with exponential growth of PCR products started to be detected by the laser detector of the ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA, USA), using PE biosystems analysis software according to the manufacturer's manuals. Both the precise amount of total RNA added to each reaction mixture (based on optical density) and its quality (i.e., lack of extensive degradation) are difficult to assess. Therefore, transcripts of the TBP gene encoding the TATA box-binding protein were also quantified as an endogenous RNA control. Each sample was normalized on the basis of its TBP content.

Results, expressed as n-fold differences in target gene expression relative to the murine and human TBP genes (both the murine and the human TBP transcripts) and termed “Ntarget,” were determined as Ntarget = 2ΔCtarget, where the ΔCt value of the sample was determined by subtracting the average Ct value of target gene from the average Ct value of murine and human TBP genes.

The primers for genes were chosen with the assistance of Oligo 6.0 software (National Biosciences, Plymouth, MN, USA). The murine target gene primer pairs and the human target gene primer pairs were selected to be unique compared to the sequences of their respective orthologous gene, whereas the total TBP primer pair was selected to amplify both the mouse and the human TBP genes. dbEST and nr databases were scanned to confirm the total gene specificity of the nucleotide

### Table 1. Predictive Gene Expression in Response to Therapies

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<tr>
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--; negative predictive value of response; +, positive predictive value of response; EMT, epithelial-mesenchymal transition; Hs, human; Mm, mouse; PUMA, p53 upregulated modulator of apoptosis.

**Histopathologic Analyses**

Various criteria have been studied, for example, the proportion of viable cells and necrosis in tumor tissue, the percentage of Ki67-positive tumor cells (anti-mouse Ki67 MIB1; Dako, Glostrup, Denmark), and the number of vessels per field (×400) in both areas of viable tumor cell and necrosis, using CD31 immunostaining (rabbit anti-CD31 Neomarkers; Fisher Scientific, Fremont, CA, USA). Consequently, two scores were defined: score 1-tumor, calculated as [% of viable tumor cells × number of vessels per field in viable tumor cell area] and score
2-necrosis, calculated as [% of necrosis \times \text{number of vessels per field in necrosis area}].

**RESULTS**

**Rb1 Molecular Analyses**

All mutations found in the patients’ tumors were correctly identified in the xenografts. Accordingly, the splice mutations c.1389+1G>A and LOH were found in both the patients’ tumors and the xenograft Rb 102, the splice mutation c.2106+2T>G and LOH in both the patient’s tumor and xenograft Rb 111, and last, biallelic promoter methylation was found in both the patients’ tumors and xenograft Rb 200. No further RB1 anomalies were detected in the xenografts.

**Bevacizumab Significantly Increases In Vivo Efficacy of carboplatin**

In the first part of our in vivo experiments, carboplatin induced a significant TGI of 72%, with complete remission (CR) in 5 of 8 patients in the Rb102 xenograft but no in vivo efficacy in either the Rb111 or the Rb200 model (TGI of 0% and 33%, respectively) (Fig. 1). Similarly, bevacizumab induced an optimal TGI of 63%, 40%, and 28% in the Rb102, Rb111, and Rb200 PDXs, respectively, without CR (Fig. 1). In contrast, the combination of carboplatin + bevacizumab was always significantly more efficient than carboplatin alone or bevacizumab alone, with an optimal TGI of 98%, 53%, and 78% in the Rb102, Rb111, and Rb200 PDXs, respectively, and 6 of 8 patients experienced CR in the Rb102 model (Fig. 1).

Hence, when we looked at individual tumor responses in the three treated models, the overall response rates (ORR), defined as an individual \(\frac{\text{TGI}}{C_0}\) value lower than \(-0.5\), were 48%, 27%, and 86% after carboplatin, bevacizumab, and carboplatin + bevacizumab, respectively (carboplatin versus carboplatin + bevacizumab, \(P < 10^{-2}\); bevacizumab versus carboplatin + bevacizumab, \(P < 10^{-3}\) (Fig. 2A–C)). When the proportion of responding treated mice (y-axis) was notified according to the quality of response (x-axis), we observed that 50% of mice (Fig. 2, dotted black line) had reached an ORR of \(\frac{C_0}{C_0} < 0.42\), \(\frac{C_0}{C_0} < 0.40\), and \(\frac{C_0}{C_0} < 0.79\) after carboplatin, bevacizumab, and carboplatin + bevacizumab, respectively (Fig. 2D). It is noteworthy that, in both the responding and PDXs refractory to carboplatin treatment, combination with bevacizumab significantly increased TGI and ORR.

In the second part of our in vivo experiments, we investigated whether the antitumor efficacy of carboplatin used at lower doses could also have been increased by bevacizumab. Carboplatin was administered at a dosage of 22 or 44 mg/kg weekly with or without bevacizumab (15 mg/kg once per week) in the Rb200 PDX. We observed that carboplatin alone induced a TGI of 48% and 64% after 22 mg/kg and 44 mg/kg administration, respectively, that bevacizumab alone induced a TGI of 38%, and that the two treatment combinations induced significantly higher TGI of
64% and 85% for carboplatin at 22 mg/kg + bevacizumab and carboplatin at 44 mg/kg + bevacizumab, respectively (Fig. 3A). This effect is particularly important at the intermediate dose of carboplatin, that is, 44 mg/kg, where the ORR lower than −0.5 and −0.75 were 30% and 10% for bevacizumab alone, respectively; 87% and 25% for carboplatin alone; and 100% and 78% for bevacizumab + carboplatin, respectively (Fig. 3B–C). In this experiment, we confirm the fact that such a combination was able to increase both proportion and quality of responses. It must be mentioned that bevacizumab induced an increased expression of the human VEGFA, the proapoptotic human gene (known to be involved in response to carboplatin), the proapoptotic human gene MYC, the proapoptotic human gene BBC3, the human VEGFA gene, and the mouse PGF gene had a positive predictive value of response, whereas the human antiproliferative human gene GADD45A had a negative predictive value (Fig. 4; Table 1). For bevacizumab therapy, the human VEGFA gene expression was shown in the responding Rb102 xenograft. After bevacizumab administration, we observed an increase in human and mouse PGF gene expression in both lower doses was also increased by concomitant administration of bevacizumab.

### Quantitative PCR Analyses

We first looked at identification of genes whose expression was correlated to in vivo response to tested therapies. After carboplatin or bevacizumab treatment, 1 PDX (Rb102) could be considered responding and the 2 others (Rb111 and Rb200) as resistant models. Hence, to define predictive markers of response to therapies, we compared basal gene expression of the Rb102 PDX versus gene expression of Rb111 + Rb200 xenografts. However, because of the high in vivo efficacy of the carboplatin + bevacizumab combination in the three tested Rx PDXs, it was not possible to define predictive markers of response after the carboplatin + bevacizumab combination. In a second trial, we looked at modifications of gene expression occurring after each in vivo therapy. We considered that the gene was differentially expressed between treatments and control groups when the fold change treatment/control of the median gene expression value obtained with the three tested PDXs was >1.5 or <−1.5, using a 2-way ANOVA test.

For carboplatin treatment, the human proapoptotic CCNE1 gene expression was differentially expressed between treatments and control groups when the fold change treatment/control of the median gene expression value obtained with the three tested PDXs was >1.5 or <−1.5, using a 2-way ANOVA test.

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<td>CXCR4 Mm</td>
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<td>CXCL12 Hs</td>
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<tr>
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<td>ICAM1 Hs</td>
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</tr>
<tr>
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<td>ESR1 Hs</td>
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+, >1.5-fold change; −, <1.5-fold change; C, carboplatin; B, bevacizumab; B + C, bevacizumab + carboplatin; EMT, epithelial-mesenchymal transition; Hs, human; Mm, mouse.
bevacizumab + carboplatin administration, we observed an increased expression of the human VEGF1 gene and a decreased expression of the human VIM gene and the mouse PGF gene in the three treated PDXs.

**Histopathologic Study**

Comparison of the three untreated Rb PDXs did not show significant differences in terms of proportion of viable cells and necrosis in tumors, percentage of Ki-67-positive tumor cells, and the number of vessels per field in both areas of viable tumor cell and necrosis (Fig. 6). However, as score 2-necrosis was not different among tumors, the score 1-tumor results were 285, 238, and 180 in the Rb102, Rb111, and Rb200 PDXs, respectively (Table 3).

The proportion of viable tumor cells and, consequently, of necrosis was not significantly modified by carboplatin, bevacizumab, or bevacizumab + carboplatin administration in either Rb102 or Rb200 xenografts; in contrast, proportion of necrosis was highly increased in the Rb111 model after carboplatin or bevacizumab treatment. However, this observation was not correlated with in vivo efficacy (data not shown). The score 1-tumor was not modified after in vivo treatments, except for the Rb200 PDX and bevacizumab + carboplatin; similarly, the score 2-necrosis was not modified after in vivo treatments, except for both Rb111 and Rb200 PDXs after carboplatin or bevacizumab administration (Table 3). The number of vessels per field × 400, which represents vessel diversity in tissues, in tumor area was not modified after in vivo treatments, except for the Rb200 PDX and carboplatin (data not shown). Finally, when studied histopathologic criteria were correlated to individual tumor response, no correlation was observed.

**Table 3.** Histopathologic Observations Under Therapies

<table>
<thead>
<tr>
<th>PDX Group</th>
<th>% Viable Tumor</th>
<th>% Necrosis</th>
<th>% Ki-67+ Cells</th>
<th>Number of Vessels in Tumor Area</th>
<th>Number of Vessels in Necrosis Area</th>
<th>Score 1 Tumor</th>
<th>Score 2 Necrosis</th>
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<td>70</td>
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<td>2</td>
<td>1</td>
<td>180</td>
<td>10</td>
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<tr>
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<td>80</td>
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<td>2</td>
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<tr>
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<td>1</td>
<td>238</td>
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<td>3</td>
<td>1</td>
<td>295</td>
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Beva, bevacizumab; Carbo, carboplatin; Combi, bevacizumab + carboplatin; PDX, patient-derived xenograft.
DiscusSion

Our in vivo study has clearly justified the interest in combining carboplatin and bevacizumab in retinoblastoma PDXs. Our results are particularly impressive due to a similar additive effect of tested combination in all three treated models. Hence, when looking at the overall response rate, combining all experiments, it was 27% and 48% after carboplatin and bevacizumab administration and 86% after treatment association (carboplatin versus carboplatin + bevacizumab, \( P < 10^{-2} \); bevacizumab versus carboplatin + bevacizumab, \( P < 10^{-3} \)). Such an observation clearly favors a clinical assessment using a systemic or a local route of administration of the studied combination, as already reported, 40 of the studied association.

In this perspective, we have also validated the fact that bevacizumab also increased efficacy of carboplatin used at lower doses, suggesting that it might be possible to decrease the cumulative dose of carboplatin and, consequently, the risk of secondary tumors with similar antitumor efficacy. Our in vivo experiments might therefore resolve a real clinical issue in the management of Rb-bearing children. Due to VEGF expression in human fetal skeletal tissues, \(^{45} \) it must be noted that long-term toxicity of bevacizumab in children should be kept in mind and complications such as potential altered bone growth must be monitored. \(^{46} \)

In this view, it is noteworthy that various preclinical assessments have previously been reported to lead further clinical trials, such as carboplatin, which was evaluated in a subconjunctival \(^{47} \) or intravitreally \(^{48-49} \) route of administration. Carboplatin has also been loaded with protein nanoparticles and tested first in Rb cells \(^{50} \) and a second time in patients. \(^{51} \) Our results and others therefore highlight the interest of relevant preclinical models that highly reproduce molecular features of human tumors. In this view, patient-derived xenografts constitute the most reliable models with which to evaluate new therapeutic compounds and new treatment combinations in the field of oncology. \(^{52-53} \) In particular, in vivo procedures for assessment of therapeutic efficacy have been well standardized and readily allow evaluation of combined therapies, especially for the purposes of objective biostatistical assessment. The use of Rb PDXs, therefore, supports our therapeutic conclusion and their clinical perspectives.

Oncologists must now define the best clinical modalities for the carboplatin-bevacizumab combination, in terms of clinical indications, route of administration (intravenous or intravitreal injections), and dosages of carboplatin. Our results open a large field of both preclinical (possibly in orthotopic models) and clinical investigations that should aim to achieve a real improvement in the management of children. Finally, considering the high efficacy of bevacizumab administered alone, various other therapeutic combinations could also be tested, such as radiotherapy or photodynamic therapy that possesses a high rational in the setting of locally advanced retinoblastoma. \(^{54-55} \)

As a confirmation of the in vivo effect of bevacizumab alone or in combination with carboplatin, we observed that the expression of genes encoding angiogenic factors were highly impacted under therapies, including the human and mouse VEGFA genes, the human and mouse VEGFR genes, and the human and mouse PGF genes. In contrast, possibly due to the limited number of included PDXs in this study, and the very high efficacy of carboplatin + bevacizumab combination, we were not able to identify predictive markers of response. However, in our study, the lack of predictive marker identification is well balanced by the very high antitumor activity of the studied treatment combination.

Overall, our in vivo results confirm the interest in antiangiogenic therapy in the treatment of Rb in combination with carboplatin and provide a robust rationale to test this combination in the clinical setting for Rb patients.

Acknowledgments

The authors thank the animal facilities team of the Institut Curie, Isabelle Grandjean, Vincent Bordier, Ahmed Rahali, and Maxime Verlhac. Supported by the Société Française de lutte contre les Cancers et leucémies de l’Enfant et de l’adolescent and Fédération Enfants et Santé.

Disclosure: F. Assayag, None; A. Nicolas, None; S. Vacher, None; C. Dechainault, None; I. Bieche, None; D. Meseure, None; I. Aerts, None; N. Cassoux, None; C. Houdayer, None; F. Doz, None; D. Decaudin, None

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