

## Topical ONYX-015 in the Treatment of Premalignant Oral Dysplasia: Another Role for the Cold Virus?

ONYX-015 IS an E1B-55kd gene deleted adenovirus which has been reported to selectively replicate in *p53* dysfunctional tumor cells.<sup>1</sup> The first clinical trial with this virus was in patients with recurrent head and neck cancer. A phase I trial in 22 patients showed no serious toxicity by intratumoral injection up to a viral dose of  $1 \times 10^{11}$  plaque forming units (pfu), with some evidence of antitumoral activity.<sup>2</sup> A phase II trial showed enhanced efficacy when the virus was given by multiple daily injections in the same group of patients.<sup>3,4</sup> In these trials, response and viral replication was correlated with *p53* status by gene sequencing and immunohistochemistry. When combined with chemotherapy, efficacy was further improved. A phase II trial of intratumoral injection of ONYX-015 combined with cisplatin and fluorouracil showed a response rate of 63%, with improved progression-free survival retrospectively compared with conventional chemotherapy alone. This suggested additive, and possible synergistic, mechanisms between these agents.<sup>5</sup> In this study, similar responses were observed in tumors with mutant *p53* (five [71%] of seven patients) and wild-type *p53* (nine [69%] of 13 patients). Selective viral replication was detected in tumor tissue with no evidence of replication in normal tissue with wild-type *p53*. Subsequent reports have questioned the selectivity of ONYX-015 by showing a lack of association with treatment response and *p53* mutational status.<sup>6-8</sup> Others have reported that *p53* function status, rather than its mutational status, is a more important predictor of ONYX-015 sensitivity, associating p14ARF and mdm2 aberrations with replication efficiency.<sup>9,10</sup> Clearly, the mechanisms of action are more complicated than initially proposed.

Despite the questions regarding ONYX-015's mechanism of activity, clinical trials in many tumor systems using a variety of administration routes have already been completed. Intraperitoneal injection of ONYX-015 in patients with ovarian cancer,<sup>11</sup> intratumoral injection of liver tumors,<sup>12</sup> intratumoral injection of pancreatic tumors,<sup>13</sup> and intrahepatic artery infusion of metastatic liver disease<sup>14,15</sup> have all been reported. A phase III, prospective, randomized controlled trial of chemotherapy alone compared with chemotherapy + ONYX-015 in head and neck cancer is currently underway.

In this issue of the *Journal of Clinical Oncology*, Rudin et al<sup>16</sup> report a novel route for administration and therapeutic application for ONYX-015. Their study assessed the feasibility and activity of ONYX-015 administered topically as a mouthwash in patients with clinically apparent premalignant oral dysplasia, a process in which progression of premalignant to invasive intraoral cancer has been reported to occur in 30% of patients over a 10-year period.<sup>17</sup> These patients are commonly treated by local excision and regular follow-up. Photodynamic therapy and laser

excisions have also been used in this setting.<sup>18-20</sup> However, due to the presence of field cancerization,<sup>21</sup> lesional destructive therapies have not been effective in long-term disease prevention. As such, there has been significant interest in the development of chemopreventative agents to treat premalignant oral dysplasia. The most frequently studied agent is 13-*cis*-retinoic acid (isotretinoin). At high doses, isotretinoin has been reported to cause histological and clinical regression compared with placebo.<sup>22</sup> However, the treatment has limited efficacy, with relapse within 3 months, and it is associated with significant toxicities such as xeroderma and conjunctivitis. Lower doses can delay progression, but not prevent the development of carcinoma.<sup>23,24</sup> Combination with alpha-tocopherol can increase response rates to 78%.<sup>25</sup> However, recurrence of oral dysplasia and the risk of subsequent progression to invasive cancer return to baseline after discontinuation of therapy.

Since head and neck cancers represent a progressive genetic disease, biologic treatment strategies have been sought. In this regard, loss of heterozygosity at chromosome 17p, the locus containing the *p53* gene, has been reported to increase with progression from mild to severe dysplasia to invasive cancer.<sup>26</sup> Moreover, *p53* protein expression status is a predictor of progression of premalignant oral dysplasias to invasive cancers.<sup>17,27</sup> Since premalignant oral dysplasia has a high prevalence of *p53* dysfunction, it would be an ideal target for ONYX-015 therapy.<sup>28,29</sup> In the study by Rudin et al,<sup>16</sup> 19 assessable patients with histologically confirmed oral dysplasia had ONYX-015 administered as a mouthwash. Three different regimens were studied. In regimen 1, the virus was given as a mouthwash at a dose of  $1 \times 10^{10}$  pfu daily for 5 days, with cycles repeated four times weekly for a maximum of 12 cycles. Two of 4 patients treated had histological resolution of dysplasia after six cycles. This was, however, short-lived, and the disease recurred in both patients. Therefore, regimen 2 employed a more frequent administration of virus at  $1 \times 10^{10}$  pfu weekly for 24 weeks. In this regimen, four of twelve patients had complete resolution. This was, again, short-lived in two patients (recurrence at 24 weeks and 48 weeks, respectively), but a durable response was observed in one patient with no recurrence at 30 months' follow-up. In regimen 3, the virus was administered to three patients at a higher dose of  $1 \times 10^{11}$  pfu daily for 5 days, followed by weekly administration for 5 weeks. In one patient, a complete response occurred that was also durable with no recurrence by 30 months posttreatment. To determine if response was correlated to *p53* function, *p53* protein expression was assessed by immunohistochemistry. A significant suppression in *p53* protein expression was reported in responders compared with nonresponders ( $P = .027$ ). No association was observed with cyclin D1 or Ki-67 expression status. Finally, to confirm the presence of viral infection, the authors

demonstrated the presence of viral replication in two of three cases tested by in situ hybridization.

This trial clearly establishes the absence of serious toxicity resulting from use of ONYX-15 as a mouthwash. Only one patient had a rising antibody titer posttreatment, indicating that systemic exposure to the virus did not occur, limiting any serious toxicity. It also shows that the virus gains entry to and replicates in the target tissue. However, the efficacy of ONYX-15 mouthwash remains to be determined. In this context, it is important to note that premalignant oral dysplasia is a condition in which spontaneous resolution can occur.<sup>30</sup> The authors found a lack of correlation between pretreatment *p53* levels and responsiveness to ONYX-015 treatment. This clearly differs from earlier work using intralesional ONYX-015 injection alone in the treatment of head and neck cancers. Combined, these factors indicate the possibility that responses observed in this study may not solely reflect the effects of ONYX-015 treatment. Moreover, since *p53* is a marker for premalignant disease, its presence is expected to decrease in response to resolution of premalignant changes. Accordingly, differences in the *p53* staining status in “responding” and “nonresponding” cases also may not directly reflect ONYX-15 activity. Nonetheless, this trial establishes the need for a larger phase II/III trial to determine the activity of ONYX-015 mouthwash in the treatment of premalignant oral

dysplasia. More detailed analysis of *p53* status is also required, such as *p53* gene sequencing, *mdm2* expression, and *p14ARF* expression. In addition, combination treatment with novel agents that act in a *p53*-independent manner should also be considered. For example, isotretinoin works most effectively in dysplastic lesions without dysfunctional *p53*.<sup>22</sup> Combination with ONYX-015 may therefore be additive due to the different mechanisms of action on tumor cells heterogeneous in *p53* status. Moreover, potential synergy may exist between the two agents since isotretinoin has been shown to reverse differentiation in hyperplastic oral lesions. This would allow ONYX-015 to penetrate the basal layer of the epithelium of dysplastic lesions more effectively. Therefore, a phase II trial in combination with isotretinoin should also be considered. Clearly, biologic therapies such as ONYX-015 are likely to play an important role in targeting progression of precancerous lesions to invasive intraoral cancer, a condition in which survival has remained static during the last 40 years, despite multiple therapeutic advances.

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#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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