The d1520 Virus Is Found Preferentially in Tumor Tissue after Direct Intratumoral Injection in Oral Carcinoma

To the Editor:

In 2003 we published two articles in Clinical Cancer Research describing the results of our clinical studies with ONYX-015 in patients with hepatobiliary tumors (1, 2). In the article by Makower et al., a phase II trial of intrallesional ONYX-015 was conducted in patients with hepatobiliary tumors to determine the safety and efficacy of such a treatment. Patients received intrallesional injections of ONYX-015 at either 6 × 10⁹ or 1 × 10¹⁰ plaque-forming units/lesion up to a total dose of 3 × 10¹⁰ plaque-forming units. Among evaluable patients, 1 of 16 (6.3%) had a partial response, 1 of 16 (6.3%) had prolonged disease stabilization, and 8 of 16 (50%) had a ≥50% reduction in tumor markers. Fifteen of 18 (83.3%) had evidence of p53 mutation. We concluded that intrallesional treatment with ONYX-015 in patients with hepatobiliary tumors was safe and well tolerated, and some patients had evidence of an anticancer effect. The high incidence of p53 mutations in these tumors precluded definitive evaluation about the necessity of a p53 mutation for ONYX-015 clinical activity.

In the second article, we hypothesized that transcription of a late viral gene, hexon, would best show replication of ONYX-015 in human tissues in vivo. Although the presence of adenoviral DNA or structural proteins such as hexon could result from the presence of the input virus, mRNA could only be present if the virus was actively replicating. A patient with paired abdominal wall implants from a primary gall bladder carcinoma was injected with ONYX-015, 1 × 10¹⁰ viral particles per lesion, followed by sequential excision of the lesions at 37 hours and 7 days. In situ reverse transcription-PCR was employed to measure expression of hexon mRNA. Strong signals were obtained in gland-forming tumor cells both at 37 hours and at 7 days, and in adjacent normal stromal cells. We concluded that evidence for transcription of hexon confirms that ONYX-015 is not only present but also capable of replicating in tumor cells up to 1 week after intrallesional injection.

Without exhaustively summarizing the results of these trials, our main findings were that (a) ONYX-015 had modest clinical activity in patients with hepatobiliary tumors; (b) there was no correlation between this activity and the presence of a mutation in p53; and (c) using in situ reverse transcription-PCR, the virus was both found and was replicating in tumor and surrounding stromal tissue at 37 hours and 7 days after adenovirus vector administration.

The results of our studies were not only published in Clinical Cancer Research but also were presented at the AACR annual meeting. The findings met with substantial resistance from investigators who seemed invested in the opposite conclusions, specifically that ONYX-015 replicated preferentially in tumor tissue but not in normal surrounding tissue, and that this was the basis for its selectivity as anticancer therapy. Thus, it was with much interest that we recently read the article by Morley et al. in Clinical Cancer Research (3). The aims of the trial were to determine whether ONYX-015 is selective for tumor tissue, whether this relates to p53 status of the tumor, and to determine level of viral spread and replication within these tissues. In addition, virus-induced apoptosis after viral injection was measured in both tumor and normal tissue. For some of these studies, the investigators employed techniques very similar to our own, including intratumoral injection, gene sequencing and immunocytochemistry for p53, and in situ hybridization for presence of adenovirus DNA. However, after reading the work of Morley et al, we wish to take issue with some of the conclusions of their article and with the omission of two references to our work. Specifically, their assays for hexon by immunocytochemistry and viral DNA by in situ hybridization cannot distinguish residual input virus from the products of viral replication.

Viral replication and survival. The authors were able to detect hexon protein by immunocytochemistry in the injected tumor tissues and concluded that this indicated higher levels of “viral survival and replication in tumor samples than in normal tissue”. Unfortunately, the presence of hexon, as described above, does not ensure that the virus is replicating. Once the virus is injected into tissues, it can remain for a prolonged period of time. Thus, immunocytochemistry may be detecting input virus and does not discriminate between input virus and replicating virus. In addition to detecting the adenoviral proteins (hexon, penton, and fiber) by immunocytochemistry in the tumor tissue described in our studies, we used viral mRNA detection by in situ reverse transcription-PCR. Viral mRNA is not present in the viral inoculum and thus is a hallmark of intracellular transcription of late viral genes. In a recent study using a nonreplicating adenovirus, the suggestion that the presence of viral genomes as detected by TaqMan assay is evidence for viral replication has been disputed (4). Specifically, among 190 subjects treated with the replication-incompetent RPR/INGN 201 adenovirus, vector-related sequences were detected in urine up to 28 days after the final injection of virus. This shows that detection of viral genome, even persistently, or a structural protein present on the infecting adenovirus is not equivalent to demonstrating viral replication.

Selectivity of virus for tumor tissue. The authors note in the abstract that “virus replicates selectively in tumor as opposed to normal tissue after this direct injection”. Whereas ONYX-015 may have some preference for tumor tissue, the authors’ data and conclusions as well as those of others (5–10) indicate that the issue is far more complex than the presence of mutated p53. Certainly, as Morley and colleagues point out, after direct injection the virus is present in normal tissues. This observation was supported by the results of our clinical trial (1, 2). Nevertheless, we find it difficult to draw any additional conclusions about viral replication and survival from the assays employed in the Morley article.

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References

In Response:
I thank the editors for the opportunity to respond to the comments made by Dr. Wadler and colleagues concerning our article, “The dl1520 virus is found preferentially in tumor tissue after direct intratumoral injection in oral carcinoma.” This article described a clinical trial whereby it was shown that the E1B-deleted adenovirus dl1520 was found preferentially in tumor tissue as compared with normal mucosa following direct injection (1).

Dr. Wadler draws attention to his article which showed viral presence in both hepatobiliary tumors and surrounding stromal tissue (2). Our results show that the virus is found preferentially in tumor tissue, although along with Dr. Wadler we were not able to show a difference in terms of p53 expression. The techniques we used included immunohistochemistry and in situ hybridization to detect adenoviral presence. Dr. Wadler’s team point out the potential advantages of using mRNA detection as an adjunct to these techniques. There may well be advantages in this where fresh as opposed to formalin-fixed tissues are concerned. We do not feel that this technical refinement invalidates our findings however, as in situ hybridization, particularly when positive at 14 days, is accepted as indicating viral replication.

Another significant difference between oral cancer and hepatobiliary carcinoma cutaneous deposits is the concept of field change. Oral carcinoma often occurs against a background of mucosal field change where genetic abnormalities, including p53 abnormalities, may be harbored (3). I wonder if the stromal tissues surrounding the hepatobiliary deposits where dl1520 was found are genetically absolutely normal?

I thank Dr. Wadler and his team for their interest in our study.

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