**Sodium Iodide Symporter SPECT Imaging of a Patient Treated With Oncolytic Adenovirus Ad5/3-Δ24-hNIS**

To the editor:

Because of the lack of curative treatment options for most advanced cancers, innovative and experimental strategies are being developed. One promising therapeutic approach is the delivery of human sodium iodide symporter (hNIS) with oncolytic viruses.\(^1\)\(^-\)\(^5\) hNIS provides the dual utility of therapy and imaging of virus distribution with radionuclides such as iodides or technetium. Although therapy is the ultimate goal, imaging of the virus is also critical because all available biodistribution information concerning oncolytic viruses is currently based on animal models, which may not be fully representative for species-specific viruses such as human adenovirus. We and others have published preclinical data on the use of radionuclide transporters as transgenes,\(^2\)\(^-\)\(^7\) and hNIS has thus far been the most popular approach. In general, two approaches have been used to arm oncolytic viruses with human NIS: replication-coupled expression and replication-independent expression. The former is useful for detection of virus replication but may not reveal overall virus biodistribution, and the opposite is true for the latter.

Utilizing replication-independent human NIS expression, Barton et al. recently published an elegant first-in-humans phase I trial.\(^1\) Nuclear imaging showed that seven of nine (78%) patients treated intratumorally with the higher viral doses and six of six (100%) treated with the highest dose accumulated technetium-99m \(^99m\)Tc. These data, together with promising preclinical data on Ad5/3-Δ24-hNIS (ref. 4) and previous clinical experience with different oncolytic adenoviruses,\(^8\)\(^-\)\(^11\) led us to treat one patient in a Finnish Medicines Agency-approved Advanced Therapy Access Program, which allows personalized patient-by-patient therapy under the “hospital exemption.”\(^12\) The patient was a 50-year-old woman with chemotherapy-refractory cervical carcinoma metastatic to the lungs, lesser pelvis, pelvic lymph nodes, liver, and bones. She had previously been treated with cisplatin, cisplatin with topotecan, paclitaxel and gemcitabine, and radiotherapy.

The patient received a total of \(3 \times 10^{11}\) viral particles into the clinically most relevant tumors—as evaluated by positron emission tomography–computed tomography (PET-CT) imaging on the same day—in the pelvis and liver. Twenty hours after treatment with virus, the patient received an injection of iodine-123 (\(^123I\)), a low-energy \(\gamma\)-emitter with excellent imaging properties that is frequently used for thyroid and brain imaging. A series of scans was performed: a 30-minute dynamic-imaging series at injection time (0 hour), whole-body single-photon emission–computed tomography (SPECT)-CT scans at 4 hours, 8 hours, and 24 hours after \(^123I\) injection. These scans did not reveal any accumulation of iodine into the injected regions over baseline readings (Figure 1a). Therefore, the imaging was repeated on the third day (68 hours after virus injection), this time with \(^99m\)TcO\(_4\) (to exclude iodide-specific issues), resulting in the same negative result. To exclude the possibility of protracted amplification of signal, one more round of \(^123I\) imaging was performed on day 6 post virus. Careful examination still did not reveal any signal enhancement other than that resulting from endogenous hNIS expression in the thyroid, stomach, and salivary glands. Urine was visualized due to renal excretion of iodide.

Blood samples were taken on days 0, 1, 2, and 6 and analyzed for adenovirus copy number with quantitative PCR.\(^9\) Before treatment, no virus was detected. However, on days 1 and 2, less than 500 virus particles per milliliter (vp/ml) and 825 vp/ml were measured, respectively, suggesting that the highest peak of virus replication occurred exactly when imaging was performed (Figure 1b). On day 6, the value had declined to 0 vp/ml. The patient reported grade 1 lower-limb edema and grade 2 dyspnea and anorexia after the treatment. No changes in laboratory values were observed. The neutralizing-antibody titer measured before treatment was 1:256 and thus only moderately elevated,\(^8\)\(^-\)\(^11\) and it rose to 1:16,384 a week after treatment. A pretreatment biopsy contained 60% poorly differentiated carcinoma, 35% fibrotic stroma, and 5% necrotic tissue (Figure 1c). Consequently, neither antibodies nor high stromal content seem likely reasons for low hNIS expression.

Our findings suggest that constructs optimized for oncolysis and featuring replication-coupled transgene expression, such as Ad5/3-Δ24-hNIS, might not be optimal for detection of hNIS expression in humans. By comparing differences in virus design and treatment protocol with Barton et al.,\(^1\)\(^-\)\(^5\) we believe that important vectorological lessons can be learned. Differences between treatment protocol, dose, and patient type may nevertheless also contribute to the findings. One difference between our construct and Ad5-\(\gamma\)CD/\(\mu\)TK\(_{38,39}\)rep-hNIS is the promoter driving hNIS expression. Ad5/3-Δ24-hNIS expresses hNIS from the native E3 promoter, which is activated by virus replication at about 8 hours,\(^4\) whereas in Ad5-\(\gamma\)CD/\(\mu\)TK\(_{38,39}\)rep-hNIS the transgene is under the ubiquitously active cytomegalovirus (CMV) promoter, which allows for immediate high-level expression of transgene in normal and tumor cells. This might result in a stronger signal when the injected lesion contains both cell types, which may be relevant in that the stromal component of tumors can vary between 5 and 95%. Moreover, the capacity of Ad5-\(\gamma\)CD/\(\mu\)TK\(_{38,39}\)rep-hNIS for expressing hNIS in normal tissues was demonstrated in dog prostates.\(^2\) Dogs are not permissive for human adenoviruses,\(^13\) and the dogs in the study did not have known prostate cancer. Also, the high early activity mediated by CMV, versus slower replication-coupled expression, might be useful in a situation where the cells have limited time to produce hNIS and concentrate radiotracers, before the cell is lysed. In this regard, the high oncolytic potency of Ad5/3-Δ24-hNIS (ref. 4) might work against imaging.

Furthermore, Ad5-\(\gamma\)CD/\(\mu\)TK\(_{38,39}\)rep-hNIS is based on dl1520, an adenoviral construct that has been safely used in many clinical trials.\(^14\) However, some
studies have suggested that the productive replication of d1520 might be attenuated even in permissive cells. Moreover, Ad5-yCD/mutTK_{381G}rep-hNIS represents a complex construct with three transgenes and three CMV promoters that together might further attenuate the replication, allowing for prolonged and enhanced transgene expression. Also, the trial patients received 5-fluorocytosine and valganciclovir prodrug therapy for 3 weeks, which might further hamper viral replication (at least in vitro), thus giving time for CMV-driven hNIS expression. However, it should be noted that the first images were captured before prodrug therapy.16 The patients also received radiation therapy, which might influence virus replication and transgene expression.

The administration technique might also contribute to the findings. Our patient received Ad5/3-Δ24-hNIS into a bulky pelvis tumor through three needle tracts and into a liver metastasis through one needle tract, as opposed to subjects in Barton and colleagues’ study2 who received the whole dose along one needle tract, resulting in a higher local virus concentration that might have facilitated imaging.

In summary, we believe our experience with Ad5/3-Δ24-hNIS in comparison with the report on Ad5-yCD/mutTK_{381G}rep-hNIS reveals several important aspects in virus design. First, low oncolytic potency may be useful for expression of transgene products located on the cell surface. Second, slower virus replication (e.g., in combination with replication-attenuating prodrugs) may allow for better imaging of membrane-associated proteins. In addition, high levels of early transgene expression may be preferable over protracted replication-coupled transgene expression. Also, tumor-selective transgene expression may lead to less robust imaging results than expression in all transduced cells. Finally, the sensitivity of SPECT may require relatively high levels of transgene expression. Thus, Ad5-yCD/mutTK_{381G}rep-hNIS may represent a useful agent for combining oncolytic virotherapy with prodrug-converting enzymes, imaging, and radionuclide therapy, whereas Ad5/3-Δ24-hNIS type viruses are more suitable for optimal oncolysis and expressing transgenes with systemic or paracrine rather than cell membrane-restricted effects. Therefore, we are currently not planning further treatments with Ad5/3-Δ24-hNIS.

CONFLICT OF INTEREST
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