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Adenovirus Gene Therapy for Pediatric Cancers: Shall We Gather at the Liver?

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Hepatoblastoma is a rare malignancy of childhood, affecting approximately 100 children in the United States each year [1]. Data collected by the United States Surveillance, Epidemiology, and End Results Program on patients diagnosed from 1972 through 1992 revealed a 5% annual increase in the incidence of hepatoblastoma, presumably due to improved neonatal outcomes for premature infants, which appears to be a risk factor [1,2].

Nearly one-third of hepatoblastoma patients present with low-risk disease (Stage I—completely resected, or Stage II—microscopic residual), for whom national and international cooperative group trials employing various adjuvant chemotherapy regimens have demonstrated an event-free survival exceeding 90% [3–6]. For the majority of hepatoblastoma patients who present with intermediate-risk disease (unresectable, Stage III) and high-risk disease (metastatic, or hepatoblastoma with initial α FP <100 ng/ml), however, historical event-free survival estimates are much worse, in the range of 50–60% [3–6].

The recent Children's Cancer Group (CCG 8881) and Pediatric Oncology Group (POG 8945) intergroup trial (INT-0098) demonstrated similar survival in hepatoblastoma patients treated with either cisplatin/5-fluorouracil/vincristine (Regimen A, C5V) or cisplatin/continuous infusion doxorubicin (Regimen B). Due to increased toxic deaths from Regimen B, the Children's Oncology Group (COG) has generally adopted C5V as standard therapy for hepatoblastoma. Re-analysis of the INT-0098 data has led to a revised definition of the potential role of doxorubicin in the treatment of children with hepatoblastoma [7]. In addition, liver transplantation has now emerged as a viable treatment option for patients with unresectable primary disease following neoadjuvant chemotherapy, which has ultimately led to improved cure rates for these patients [8]. Novel approaches to local control, including trans-arterial chemoembolization (TACE) [9] and radiofrequency ablation (RFA) [10], have shown promise in select patients. Among additional aims, the COG has considered plans to investigate (i) whether adding doxorubicin into the C5V regimen will improve the outcome for intermediate and high-risk patients, (ii) if it is feasible to expedite liver transplant consultations for appropriate patients, and (iii) whether the response rate to chemotherapy can be improved by incorporating an irinotecan/vincristine up-front window for patients with metastatic disease.

Despite intensified chemotherapeutic and advanced surgical approaches, cure remains elusive for a subset of children with hepatoblastoma. Furthermore, the need for treatment options that minimize therapy-related morbidity and late effects, expected in this vulnerable population from platinum, doxorubicin and liver transplantation, cannot be underestimated.

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One avenue for discovering new therapies is the identification of molecular targets for which there are available drugs [11–13].

Another approach to finding new hepatoblastoma treatments might be through the use of viruses. Virotherapy strategies under development for cancer include the use of viruses as oncolytic agents or simply as therapeutic gene transfer vectors. Oncolytic viruses are attenuated by genetic mutation to be safe, akin to live virus vaccines, but retain their ability to replicate within and kill cancer cells. The COG is moving forward with plans for a Phase I investigation of the oncolytic picornavirus, Seneca Valley Virus (SVV-001), in pediatric patients with relapsed/refractory solid tumors, particularly those with neuroendocrine features that appear to be most susceptible to SVV-001 infection [14]. For a historical perspective on the use of oncolytic viruses, please see the review by Kelly and Russell [15].

Viral-mediated gene transfer in the absence of a lytic virus infection also remains an active area of research, for the treatment of both single gene disorders and for cancer. In 2008, several patients with Leber's congenital amaurosis were reported to be cured by gene therapy [16], which generated considerable excitement and consolidated earlier successes using virus-mediated gene transfer to treat adenosine deaminase deficiency severe combined immune deficiency [17,18] and chronic granulomatous disease [19]. Such accomplishments have not been achieved without complications and controversy [20], but do underscore the reality of actual medical therapy using genes. Admittedly, these diseases may be more suitable targets than cancer, especially from a technical perspective as the latter two examples used gene transfer in hematopoietic stem cell progenitors after their removal *ex vivo*, which is arguably easier than delivery of virus to a mass of cancer cells *in situ*. Still, the often-heard criticism that "no one has been cured by gene therapy" is *passé*; gene therapy is no longer science fiction.

In this issue of *Pediatric Blood & Cancer*, Warmann et al. tested the ability of adenovirus to deliver an enhanced version of a hybrid prodrug enzyme to hepatoblastoma cells in order to metabolize inactive chemotherapy agents into their active metabolites within tumor cells. The goal of this type of gene- or viral-directed enzyme prodrug therapy (GDEPT or VDEPT), also known as "suicide gene therapy," is to produce relatively high drug levels within the tumor microenvironment without concurrent toxic systemic levels, theoretically improving the therapeutic index. GDEPT has been pursued since the early days of gene transfer using the herpes simplex thymidine kinase gene in conjunction with acyclovir or its analogs [21]. The idea has continued to generate significant interest for pediatric brain tumors, sarcomas, and neuroblastoma, and the technology has been refined by the development of other prodrug/enzyme combinations and methods for gene delivery [22–24]. Clinical trials have shown viruses can be given to pediatric patients safely [25], but there have only been faint hints of efficacy, as viral gene expression appears to often be limited by the anti-viral immune response [26–30]. Improved efficacy could potentially be garnered by more potent and rapid antitumor effects, occurring prior to viral clearance.

The work by Warmann et al. represents another refinement of GDEPT using a hybrid enzyme and the data look promising. They were able to show effective, rapid destruction of several hepatoblastoma cell lines when virus was combined with prodrug. Several issues warrant additional attention including the best choice of promoter (the CMV promoter was inactive in one of the cell lines), modes of delivery, as well as validation of early findings in primary hepatoblastoma cells and animal models. Nevertheless, a logical next question is: could GDEPT actually be used in patients with hepatoblastoma? If so, when?

Adenovirus is the most commonly used vector to deliver therapeutic genes into cells and patients, but it has a long and sordid history when it comes to the liver. The liver, as a

dominant blood filter, soaks up the vast majority of systemically administered adenovirus [31]. In fact, liver toxicity from adenovirus exposure was the seminal event that jolted the pursuit of gene therapy from an arguably naive if not cavalier field [32] into the more serious, thoughtful, and highly regulated endeavor it is today. Many people originally believed such liver toxicity marked the end of adenovirus as a gene therapy vehicle. Not true. Studies of attenuated adenovirus have continued unabated, and multiple clinical trials have suggested they can be used safely in humans at high doses, including newer generation viruses able to replicate and amplify their genomes in cancer cells [33]. All that said, perhaps the virus' propensity for uptake in the liver can even be an advantage for targeting diseases such as hepatoblastoma.

A search of clinicaltrials.gov on February 6, 2009 for "adenovirus" and "interventional" and "cancer" revealed 79 open clinical trials. Most of these trials are early phase (I or II) tests, either using the virus as a gene delivery vector or as an oncolytic agent. Of note, two projects have advanced to the phase III stage. A prostate cancer study seeks to determine the efficacy of adding a single intratumoral injection of adenovirus to standard radiation therapy (clinicaltrials.gov identifier #NCT00583492). In that study, the virus is an oncolytic version (conditionally replication competent), but also expresses the yeast cytosine deaminase gene similar to the one used in the Warmann study. Presumably the addition of a prodrug will be added in a later clinical trial. A second study in patients with pancreatic cancer tests the combination of radiation therapy and 5-fluorouracil with five weekly intratumoral injections of an adenovirus expressing the TNF- α gene whose expression is under the control of a radiation-inducible promoter (#NCT00051467). Although the trials are ongoing and the results pending, their mere existence implies the field of virotherapy has grown considerably over the past decade, with any perceived hurdles regarding testing viruses as new cancer therapy proven to be surmountable.

The successes already in hand for single gene disorders and the ongoing clinical trials in cancer give us hope for future FDA approval of virus-based therapies for cancer. As is usually the case with new technologies, the vast majority of effort to date has been directed at diseases that occur almost exclusively in adults. While new ideas are often tested in adults first, the unique biologies of childhood cancers dictate the need for pediatric-specific innovative approaches. Discovering and developing new therapies for childhood cancers require the significant investment of both human and financial capital specifically into studying pediatric diseases. The pace of discovery is dictated by the time, energy, and money invested. For Warmann et al.'s initial efforts to bring to bear the realm of virus therapy to the most common liver cancer of childhood, we sing their praises.

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