tumor tissue in the brain. Although such a high frequency of repetitive administration might arouse some translational concerns due to the possibility of generation of antibody to the peptide-coated NWs or liver and kidney toxicity due to nonspecific uptake, this result was particularly impressive in view of the fact that these tumor cells have been reported to differentiate into endothelial cells in a VEGF-independent manner in this GBM model.[5] Thus, the tumor was potentially resistant to VEGF-targeted antiangiogenic therapy.

The authors also tested a transplantable tumor model—the GBM 005 cell line—that was generated by a lentiviral vector expressing the oncogenes H-Ras and Akt and by knocking down p53. Although the administration of the NWs substantially prolonged survival, the treatment was noncurative in this setting. Despite the complete dysfunction of blood vessels observed from the tumor tissue harvested at the end point, the mice died. The authors speculated that fluid-conducting channels lined by tumor cells could provide blood supply to maintain tumor progression. It is also reported that GBM 005 cells contain tumor-initiating stem cells with extremely high tumor-forming ability. Only 100 cells are sufficient to form a tumor in the brain of nude mice.[6]

A similar result was observed in another orthotopic model generated by the injection of U87 human GBM cells, which also contain tumor-initiating cells with the ability of self-renewal and multipotency.[7] Taken together with the results in the first model, the findings clearly suggest that a single antiangiogenic therapy is not sufficient to eradicate GBM, particularly if tumor-initiating cells are present. Although the authors attempted to enhance the therapeutic efficacy in the GBM 005 model with the aid of an iRGD peptide, which has been shown to facilitate tumor penetration of the NWs, only prolonged survival was achieved.[8] This result is understandable, since the primary target cells of the NWs were the endothelium with the binding moieties presented as entries to the cells. Without endocytosis, the peptide is nontoxic to the cells. Therefore, the extravascular NWs in the tumor tissue were less effective in killing tumor cells, consistent with *in vitro* results showing that the IC_{50} of NWs for GBM cells (T3 and U87) was three times that for human umbilical vein endothelial cells.

In summary, Agemy *et al.* have successfully assembled multiple elements into a single theranostic NW without compromising their individual functions. The NWs could effectively target tumor vasculature and kill the endothelium with a proapoptotic peptide. Although the NWs showed exceptional efficacy in eliminating a lentivirus-induced GBM model, which is potentially refractory to antiangiogenic therapy targeting VEGF-VEGFR, it could not completely inhibit GBM growth induced by orthotopic inoculation of a GBM cell line. Although all three models share common characteristics of GBM, such as hypervasculature, the latter two tumor models contain tumor-initiating cells in the cell culture, suggesting a possible mechanism for resistance to antiangiogenic strategies in treating GBM. There is little doubt that the targeted NWs hold promise for treating brain cancer in an antiangiogenic therapy. However, it is also noteworthy that tumor-initiating cells are the evil roots that need to be taken care of for the eventual cure of GBM.

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Live and Let Die: A New Suicide Gene Therapy Moves to the Clinic

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The concept of suicide gene therapy dates from the beginning of the field of gene therapy and was one of the first clinical applications. Initially described as an anticancer therapy, it quickly found a specific application in the practice of hematopoietic stem cell transplantation. A recent publication by Di Stasi *et al.* presents the first clinical data on a relatively new suicide gene system that targets caspase 9–mediated apoptosis in an inducible fashion.[1] Specifically, five children undergoing haplo-identical stem cell transplantation for leukemia received donor T lymphocytes that had been genetically engineered with the inducible caspase 9 (iCasp9) gene. Four of the five patients developed graft-vs.-host disease (GVHD) caused by the donor lymphocytes that was quickly reversed by induction of the iCasp9 suicide gene. These results further validate the findings of others that suicide gene therapy for the control of GVHD is a valuable clinical procedure and, in the broader context, may have implications for a variety of other gene therapy applications in which a suicide safety switch may be useful.

In 1986, Moolten was the first to demonstrate that transfer of the herpesvirus thymidine kinase (HSV/Tk) gene to tumor cells could lead to their destruction

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both *in vitro* and *in vivo* following administration of a prodrug.[2] The initial reports of suicide gene therapy all involved the expression of a heterologous enzyme that could metabolize an inactive prodrug into a cytotoxic derivative leading to cell death. The most extensively studied suicide gene system is the combination of HSV/Tk and the prodrug ganciclovir (GCV). HSV/Tk has a high affinity for GCV, which allows it to catalyze the phosphorylation of GCV into its monophosphate form, which is further converted into its di- and triphosphate derivatives by cellular kinases. DNA polymerase then incorporates GCV-triphosphate into replicating DNA, leading to cell death by polymerase inhibition and induction of apoptosis. In the clinical setting, HSV/Tk-GCV was first used in an attempt to treat ovarian cancer and central nervous system malignancies via *in situ* transduction (for a concise review of cancer applications, see ref. 3). Although associated with manageable toxicities, the application of the HSV/Tk system in cancer resulted in only a limited number of objective responses. Other suicide gene systems include cytosine deaminase–5-fluorocytosine, cytochrome P450–ifosfamide, cytochrome P450–cyclophosphamide, and nitroreductase–5-[aziridin-1-yl]-2,4-dinitrobenzamide.

The most clinically efficacious application of suicide gene therapy has been in the context of controlling GVHD in allogeneic hematopoietic stem cell transplantation (allo-HSCT). Allo-HSCT is now being applied as a viable treatment option for a variety of hematological malignancies (as well as other diseases) and works by exploiting the exquisite ability of T cells to recognize and destroy cells expressing non-self antigens, in what is termed the graft-vs.-tumor effect (these cells also control infectious agents). In the case of allo-HSCT, donor-mediated graft-vs.-tumor effect can also be associated with off-tumor GVHD, which is a serious life-threatening complication in a high percentage of cases. The principle here is simple: if GVHD develops, you kill the T cells via the suicide gene. Bonini *et al.* were the first to report successful management of GVHD by cells engineered with the HSV/Tk gene (cells also coexpressed a cell surface marker protein used for physical selection).[4] In a recent summary of 123 patients treated using this approach, 23 patients who developed GVHD were successfully treated by administration of GCV, and this gene therapy has now moved into a large-scale phase III clinical trial.[5] So, if HSV/Tk-GCV is working, why fix it?

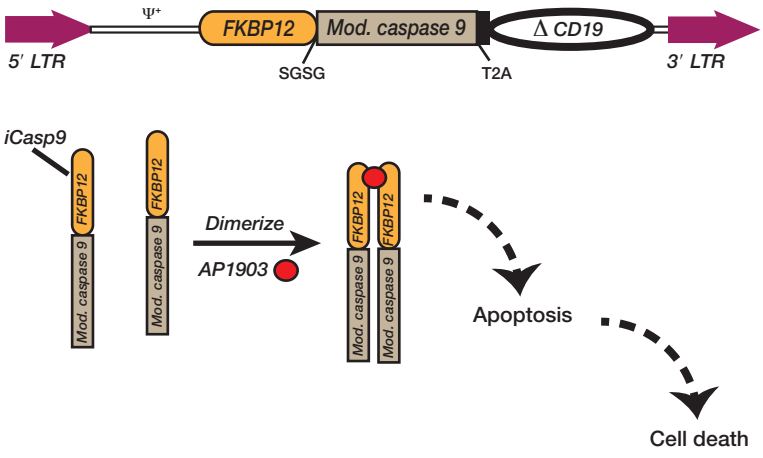

Figure 1 iCasp9 suicide gene therapy system. (Top) Diagram of the gamma-retroviral vector designed to express the iCasp9 suicide gene therapy proteins. The vector uses the viral LTR to drive the expression of a fusion protein containing a mutated (F36V) FKBP12 protein linked (SGSG) to the modified caspase 9 (deleted for activation and recruitment domains). To allow physical isolation of the iCasp9-engineered T cells, the last element in the vector is a truncated version of the human CD19 B-cell surface marker (intracellular signaling domains were removed), which can be used to isolate the engineered T cells with readily available clinical reagents. The ΔCD19 is linked to iCasp9 via a picornavirus 2A peptide (T2A). (Bottom) Biochemically, the iCasp9 fusion protein induces cell apoptosis and death only after it has been dimerized by addition of the small molecule AP1903.

The limitations of the HSV/Tk-GCV system include (i) the potential for production of inactive catalytic molecules due to the utilization of alternative splicing sites, (ii) the potential immunogenicity of the viral enzyme, (iii) the potential need to administer GCV to control cytomegalovirus infections (a complication often encountered in allo-HSCT) and thus cause unintended elimination of HSV/Tk-engineered cells, and (iv) the requirement for active cell division in order to mediate cell death, which takes time and renders the system less effective for use in postmitotic cells.

As with the latest iPhone, iCasp9 represents more than a simple upgrade of the HSV/Tk-GCV system (**Figure 1**). Proteolytic activation of caspase 9, a late executor of the intrinsic pathway of apoptosis, leads to DNA fragmentation and rapid cell death. A synthetic version of caspase 9 was created that is expressed as a pair of inactive subunits, linked to the FK506-binding protein FKBP12 (although both proteins contain mutations to increase reactivity and specificity, they are of human origin and thus theoretically less immunogenic) to yield iCasp9. The individual subunits of iCasp9 do not induce apoptosis. Dimerization of the subunits is induced by addition of a biologically inert small molecule (AP1903) that has been shown in clinical studies to be well tolerated. Dimerization of iCasp9 activates one of the last steps in the apoptotic cascade, resulting in rapid cell death—as soon as 30 minutes after administration of the activator. Most important, iCasp9-mediated cell death is not dependent on DNA synthesis as is HSV/Tk-GCV, allowing application in nonreplicating cells. As with the HSV/Tk system, the coexpression of a cell surface protein (here a truncated CD19 molecule) allows for physical enrichment of transduced cells.

In the study by Di Stasi *et al.*, five patients (aged 3, 4, 6, 8, and 17 years) received CD34-enriched haplo-identical transplants, followed 1–3 months later by infusion of the iCasp9-engineered donor T cells.[1] iCasp9-engineered T cells were detected in the circulation within 7 days after infusion (one patient received two infusions). In four of the patients, a rapid increase in iCasp9 T cells over the following week was associated with symptoms of GVHD (skin rash and,

in one patient, elevation of liver enzymes). One dose of the dimerizing drug AP1903 was administered to these patients, and this was associated with a remarkable reduction in iCasp9-engineered T cells: a 90% decrease measured by both fluorescence-activated cell sorting– and polymerase chain reaction–based assays within 30 minutes (and another 0.5- to 1.0-log decline over the next 24 hours). More significantly, in each patient treated with AP1903, symptoms of GVHD began to resolve within a day and no subsequent GVHD events were reported for the duration of the study (up to 1 year post-treatment). AP1903 did not eliminate all the iCasp9-engineered T cells; cells returned to the circulation within a week in some patients, and these cells could still be detected as long as 1 year post-treatment. Other significant elements in this report include the control of viral infections by the iCasp9-engineered T cells, the finding that cells that recovered after the first exposure to AP1903 were still sensitive to the drug months later, and the clinical response (four ongoing complete responders and one death from progressive leukemia).

Clinical reports of small numbers of patients must always be interpreted with extreme caution, and this report will need to be validated by larger studies. Consistent with the Baylor group's previous report, iCasp9 cells were not completely eliminated by AP1903 administration.[6] This observation may be related to the biology of T cells that "cool down" *in vivo* following *ex vivo* activation and the known property of the vector long terminal repeat (LTR) to respond to the state of T-cell activation.[7] In the case of GVHD, the clinician wants to eliminate highly activated alloreactive T cells while retaining virus-specific cells, so iCasp9 may be well suited to this application.

Gene therapies using integrating vectors are fundamentally different from other forms of pharmaceutical intervention in that these "living" therapies are capable of not only self-replication but also tremendous cell expansion. This amplification is the goal when one is trying to reconstitute the immune system of a patient with severe combined immunodeficiency (SCID) or to eliminate the last cancer cell in a melanoma patient, but in rare cases this comes at a price. Patient deaths have resulted from insertional mutagenesis (and resulting cellular transformation) in gamma-retroviral vector–mediated gene transfer to hematopoietic stem cells, and unchecked cytokine production has been observed in patients receiving T cells retargeted to tumor antigens.[8–10] Those engaged in gene therapy research would love to have an innocuous safety switch so that engineered cells could be eliminated if desired, but both the HSV/Tk-GCV and iCasp9 suicide systems have limitations.

These suicide genes are actually complex expression cassettes that cannot be simply inserted into another vector because of packaging size constraints and potential negative effects on vector titers. Therefore, the most effective means of high-efficiency transfer of the suicide gene will probably require cotransduction with the therapeutic vector. Not only does this require a more complex cell production system (as well as the associated costs of another vector), but it will, by definition, result in additional genomic alterations following suicide vector integration. It may therefore be prudent to consider each case individually to determine the relative merits of adding a suicide gene vector to the therapeutic vector. For example, although insertional mutagenesis has been reported in X-linked SCID, it has not been observed in patients treated for adenosine deaminase deficiency (ADA)-SCID using nearly identical vectors and cell production methods. Adding a suicide gene to ADA-SCID patient treatment might actually increase the danger of unforeseen events.

After decades of dedicated research, gene therapy has started to achieve clinical success in diseases as diverse as blindness and cancer.[11] The report by Di Stasi *et al.*[1] is an excellent example of how medical researchers can address an unmet clinical need using gene transfer technology.

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