

CD4<sup>+</sup> and CD8<sup>+</sup> T cells.<sup>17</sup> Finally, in addition to improving efficacy, a major barrier to the development of cancer vaccines has been related to the economics of delivering personalized medicine. The next generation of designer DCs are attractive in that they have the potential to provide shortcuts to the *ex vivo* manufacturing procedure, so that it may be possible to simplify the complex process currently required for production of natural DCs. Further progress in designer DCs has the potential to advance the feasibility of developing effective vaccines for several cancers and chronic infections.

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# I Could Die for You: New Prospects for Suicide in Gene Therapy

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**M**olecular therapy in the year 2050: the editor invites authors to submit papers that describe the transfer of complex genetic information containing complete sets of developmental instructions for the control of cell fate *in vivo*. By this time the technological basis for such multigene transfer will have been established, with the creation of new generations of versatile, receptor-targeted, episomal gene vectors characterized by a large genome capacity and persistent gene expression. Safe and efficient delivery of genetic information into cells no longer represents a limitation. Systems biology will have allowed more precise predictions of cell behavior in multicellular organisms and of gene interactions in response to environmental stimuli. A remaining challenge at this (unfortunately still distant) stage of the development of the field is the design of “intelligent episomes” that comprise entire gene networks that allow multimodular manipulation of cell behavior. Among the modules used for this purpose are several that fulfill only one aim: to cause the engineered cell

to self-destruct in case of misbehavior. A gene encoding the melancholic message “I could die for you,” or, in less poetic terms: targeted cell death or conditional suicide. This is not pure science fiction. Certainly some of the selectable marker genes used in the year 2050 will rely on discoveries made today.

Although suicide gene therapy has made its way into clinical trials beyond phase I/II<sup>1–4</sup> and has also been considered as an emergency brake in the case of tumor induction by insertional mutagenesis,<sup>5,6</sup> the perfect suicide gene has yet to be identified. The therapeutic value of such a gene is based on many parameters: it should not alter cell growth in the absence of a suicide-inducing drug, it should not induce the expression of immunogenic epitopes, and the drug used to trigger cell death should not be toxic in other cells. A possible breakthrough toward this goal is presented in the article by Medin’s group published in this issue of *Molecular Therapy*,<sup>7</sup> and it is quite possible that the work described in this article will continue to be referenced in manuscripts submitted in the year 2050 that describe work using complex episomes for the control of cell behavior.

So what did Medin and colleagues do? They considered that the prodrug 3′-azido-3′-deoxythymidine, better known as AZT and widely used in HIV therapy, must be converted to the toxic triphosphate AZT-TP by cellular enzymes for it to wreak its havoc on the cell. The rate-limiting step in this process is catalyzed by thymidylate kinase (TdpK). To en-

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hance the potency of the drug, Sato *et al.*<sup>7</sup> designed point mutants into the *TmpK* gene so as to accelerate AZT phosphorylation, thus efficiently converting this fairly safe prodrug into a strong antimetabolite that effectively kills cells into which it is introduced. Subsequent mechanistic studies indicated that cellular apoptosis was triggered in response to expression of the mutant *TmpK* and exposure to AZT by activation of caspase-3 and disruption of the mitochondrial inner membrane potential.

An interesting aside to this finding, as suggested by the authors, is that it may provide an explanation for the toxic mitochondrial myopathy observed in some patients subsequent to long-term treatment with AZT. Another important observation of this study is the finding that this mechanism of cell death did not necessarily require cell division. This may be of particular importance when attempting to destroy quiescent tumor stem cells or other transplanted cells that may cause harm in the body even in the absence of a high proliferative index. Underlining the potency of the mutant *TmpK*/AZT suicide team, proof-of-concept experiments revealed efficient cell death in transduced immortal and primary T cells studied *in vitro* and a transduced K562 cell tumor model evaluated in immunodeficient mice. This suggests that this particular suicide gene/prodrug combination could be useful to eliminate either transplanted T cells in case

of an attack against healthy tissue (so-called graft-versus-host disease) or tumor cells (that may arise as a complication of cell therapy or that may be directly targeted *in vivo*).

Of course, a few questions remain unanswered, but these are probably the subject of ongoing investigations. These include the long-term effects of ectopic expression of mutant *TmpK* on cell growth and differentiation, the degree of bystander killing of neighboring cells that do not contain the transgene, the ability to counteract acute and chronic graft-versus-host disease subsequent to genetic modification of T cells, the ability to enrich cells coexpressing mutant *TmpK* and a positive selection marker using clinically applicable selection systems, and the frequency of genetic and epigenetic revertants or other forms of intrinsic resistance (which have been described using other suicide genes).<sup>5,8,9</sup> Indeed, the fact that a single point mutation is sufficient to create a suicidal (and potentially nonimmunogenic) version of *TmpK* implies that another counteracting single point mutation might lead to loss of its suicide activity. A malicious “I will not die for you after all” may be the unfortunate message encoded by such an escape mutant. As suggested in the present and earlier reports,<sup>5,10</sup> this potential drawback could be addressed by the coexpression of a second suicide gene allowing targeted cell elimination. Nevertheless, the study by

Sato *et al.* introduces a promising new approach for targeted induction of cell death with proof-of-concept analyses in model systems. The mutant *TmpK*/AZT suicide combination may thus prove to be a useful new tool for future gene and cell therapy.

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