efficient in penetrating tumor tissues as compared with antibodies, and the results confirm that only three injections of T cells are necessary for complete tumor regression. However, only a comparative study using antibodies and engineered T cells will validate this claim. Patients treated with CAR-engineered T cells could also develop resistance similar to that in lymphoma patients in whom CD20 molecules were downmodulated after treatment with rituximab, rendering the antibody treatment ineffective.[19]

Finally, targeting only a minor subpopulation and leaving behind the bulk of the tumor does not take into account the dynamic nature of tumor cell subsets and the possibility that other minor subpopulations may also have tumor-initiating capabilities.[5,20] Moreover, could cells that initially do not express surface markers such as CD20 become CD20$^+$ and acquire stem cell–like properties under the influence of therapy or the tumor microenvironment? Although Schmidt and colleagues propose a novel (and possibly efficient) approach to targeting a minor subset of tumor-initiating cells, a two-pronged approach will most likely be necessary to cure melanoma. This approach should target both the large bulk of highly dynamic and proliferative tumor cells and the phenotypically distinct minor subpopulations. Future studies will be required to validate the strategy proposed by Schmidt and colleagues, its therapeutic impact, and the potential it creates to offer more effective treatments for melanoma patients.

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Nondividing Cells: A Safer Bet for Integrating Vectors?

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Most integrating vectors used in gene therapy insert their DNA in actively transcribed, gene-rich regions, a feature that increases chances of adverse events developing after vector integration. In this issue of *Molecular Therapy*, Bartholomae and colleagues report that lentiviral vectors integrate less frequently in actively transcribed genes of postmitotic neuronal and retinal cells in rodents than in rapidly dividing cells.[1] This may be good news for researchers developing treatments for disorders of these cell types because it could mean a lesser likelihood of genotoxicity following gene transfer. Bartholomae *et al.* also show that low levels of expression of the integration tethering protein LEDGF was associated with reduced integration in genes, as has been seen in human cells.[2]

Two main classes of integrating viral vectors are used for gene therapy: adeno-associated viruses and retroviruses. Adeno-associated viruses have a near-random pattern of integration with a weak tendency to favor integration within genes[3] but are less efficient at integration and can carry only small transgene cargos compared with retroviruses. Of the retroviruses, the lentivirus family offers an attractive means of gene delivery because such viruses can transduce nondividing cells and allow access to a wider array of tissues than with the earlier generation of gamma-retroviral vectors. HIV-based vectors have recently been used successfully for human gene correction.[4,5]

It is not clear why HIV does not cause cancers in humans by insertional mutagenesis—there are several types of cancer associated with HIV infection, but the transformed cells do not harbor

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integrated proviruses, ruling out insertional activation. Lentiviruses integrate throughout the length of transcription units and do not favor integration near transcription start sites (TSSs) or CpG islands, as do the gamma-retroviral vectors used in many of the first human gene therapy trials. Perhaps this partly explains the lack of insertional activation of proto-oncogenes, but other possibilities exist and may even be more likely. For example, lentiviruses are cytostatic (the *vpr* gene arrests the cell cycle) and cytopathic (*env* expression is toxic). In addition, the terminally differentiated status of cellular targets for HIV infection may limit transformation. However, the fact that lentiviruses target actively transcribed genes is probably not ideal for maximizing vector safety. This idea is reinforced by recent experience in β-thalassemia gene therapy using a lentiviral vector in humans, in which insertion of the vector within the transcription unit of the proto-oncogene *HMGA2* was associated with upregulation and clonal expansion,[4] though to date the patient is doing well.

In the study presented by Bartholomae *et al*., the integration site distribution of a self-inactivating lentiviral vector was investigated in postmitotic eye and brain tissue in rodents transduced *in vivo* and compared with sites from actively dividing fibroblast (SC-1) and hematopoietic progenitor cells transduced *ex vivo*. In the actively dividing cells, integrated vector distributions matched the expected pattern-integration sites accumulated preferentially in genes and actively transcribed regions but not near CpG islands or TSSs. Relative integration frequency in genes of postmitotic neuronal and retinal cells, however, was reduced nearly 30% in both rat and mouse samples. To investigate whether this reduction was due to fewer expressed genes present in these nondividing cells, transcriptional profiling was carried out on both dividing and nondividing cell types. The number of expressed genes was judged to be similar for both, however, suggesting that the cause of reduced integration in transcription units lay elsewhere.

Previous work has shown that the cellular transcriptional mediator protein LEDGF (product of the *PSIP1* gene) binds tightly to integrase and to chromatin, thereby increasing the efficiency of integration and targeting integration to transcription units (refs. 2, 6–9 and reviewed in ref. 10). Bartholomae *et al*. compared levels of LEDGF expression and found levels to be higher in dividing cells and lower in nondividing neuronal and retinal cells, potentially explaining the lower levels of integration in transcription units.[1] However, the authors point out that although integration within genes of the postmitotic cells mirrors observations in LEDGF knockout or knockdown cells, it does not fully match other changes observed under LEDGF-depleted conditions, specifically increased integration near CpG islands and TSSs. It is possible that these effects were not detected because of the small size of the integration site data sets studied. Bartholomae and colleagues, however, reasonably suggest that the patterns observed here may result from a combination of reduced LEDGF expression, cell status, and other undefined host factors. As the authors point out, results in studies of nondividing human cells (arrested IMR90 cells or macrophages[11,12]) did not show the large reduction of integration in transcription units observed in the rodent neuronal and retinal cells studied here. Thus, it appears that reduced frequency of integration in transcription units is not a general property of nondividing cells, although whether LEDGF levels are the full explanation is uncertain.

Reducing the proportion of vector integration in genes is important for increasing the safety of gene therapies and may translate to reduced chances of adverse events downstream. Because cellular transformation usually results from more than one genomic insult, the probability that a cell will turn cancerous may be the product of the individual probabilities for each genetic change. A linear reduction in the occurrence of one event, such as vector insertion near an oncogene, may therefore translate to a linear reduction in the overall probability of transformation. Thus, gene correction under conditions that favor targeting away from genes may improve the safety of lentiviral-mediated gene transduction. Methods for this include LEDGF knockdown, treatment with small-molecule inhibitors of integrase-LEDGF interactions ("LEDGINs"[13]), or introduction of chimeras composed of LEDGF integrase-binding domains fused to alternate chromatin-binding domains that program integration outside of transcription units.[14–16] Simplest of all, of course, is to target cell types where integration near genes is naturally minimized. If human cells parallel rodent cells, then the findings of Bartholomae and colleagues represent encouraging news for researchers working with diseases of postmitotic neurons and retinal cells.

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