(iii) stress the importance of understanding of the fundamental biology of AAVs for vector engineering. Although all the points described in these studies are positive and noteworthy, and answer the intriguing question of how big genes can effectively be delivered while still obeying the packaging constraints of AAVs, we must remember that the transition of partial AAV genome vectors toward clinical applications poses further challenges. For example, the efficiency to reconstruct the desired product from genome fragments is controlled by host cell recombination machinery that will be different for certain disorders (e.g., cystic fibrosis vs. cancer) and, more concerning, may vary from patient to patient. Then there are the never-ending technical issues when trying to "scale up" large-gene vectors for human experimentation, as a result of the inherently lower titers. Finally, vectors produced in this manner are packaged with unknown heterogeneous DNA sequences (depending on the site of genome truncation or deletion), which create additional hurdles for approval by the US Food and Drug Administration.

Despite these limitations, these three reports provide a uniform understanding pointing to a likely mechanism for successful performance of AAV "little vector, big gene transduction" experiments and highlight how these new reagents can continue to be an invaluable tool for the understanding and development of more efficient AAV vectors that can package desirable larger genomes for human therapy.

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Stem Cell Vaccination Against Cancer: Fighting Fire With Fire?

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A recent report[1] suggests yet another application for embryonic stem (ES) cell technology: using such cells to immunize against cancer. However, before this novel strategy can prove useful, several issues need to be resolved. ES cells derived from preimplantation embryos[2] and induced pluripotent stem (iPS) cells obtained by transient overexpression of specific transcription factors in somatic cells[3–5] are frequently said to hold the potential to revolutionize biomedical research by providing new substrates for biological studies designed to understand human diseases, by offering cellular transplantation and replacement therapies, and by serving as a platform for pioneering drug development and screening efforts. However, as the history of biomedical research has made clear, the most triumphant breakthroughs have often emerged from areas initially not considered promising by most researchers.

Could it be, then, that this recent report[1] by Bei Liu, Zihai Li, and others from the University of Connecticut Stem Cell Institute represents such a contemporarily disparaged yet potentially promising application of stem cell technology? The jury is still out on this question; however, it is worth carefully examining the experiments presented by these authors and contemplating their meaning.

One of the most auspicious, yet challenging, avenues for combating malignancies is to enlist the immune system to come to the defense of the patient. However, myriad components of the immune system interact in extraordinarily complex ways with active or dormant neoplastic cells, an interaction matrix that is incompletely understood at best. Nevertheless, it is apparent that the emergence of disease must be at least partly the consequence of both a failure of the immune system to respond adequately and the evolution of escape mechanisms by malignant cells.

In their article,[1] Li *et al.* reason that exposure of the immune system to novel tumor-associated antigens might boost an otherwise inadequate immune response into an effective antitumor action. What distinguishes the study is the source of these tumor-associated antigens: human ES and iPS cells. Specifically, the study investigated whether vaccination of mice with human ES or iPS cell lines would trigger an enhanced immunological response against shared

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antigens expressed by the primitive normal cells and the colon carcinoma cell line CT26—so-called oncofetal antigens. The authors report that vaccination of mice with the human ES cell line H9 induced both strong cellular and humoral immune responses against CT26 colon carcinoma that was manifested as a retardation of proliferation after injection of the cells into the experimental animal. On a more mechanistic level, they found that the protection correlated with an expansion of tumor-responsive and interferon-γ-producing cells and a profound loss of $CD11b^{+}Gr\text{-}1^{+}$ myeloid-derived suppressor cells in the spleen. Importantly, they found no evidence of any significant autoimmunity. Finally, the authors compared the immunogenicity of their human ES cell line against CT26 colon carcinomas with that of a newly established iPS cell line. Curiously, they obtained evidence that suggests that the iPS cell line was inferior to the human ES cell line in conferring tumor protection. By way of explanation, they proposed that oncofetal antigens were differentially expressed in human ES and iPS cells.

Three aspects of this study seem to deserve particular attention. The first is the concept of tumor stem cells. The authors reason that if the cancer stem cell concept is valid, then immunization with stem cells should prove valuable. However, it is possible to argue that human ES cells as a stem cell entity are defined merely on the basis of functional characteristics observed *in vitro*[6] and that they are not likely to represent authentic stem cells found during embryogenesis or in adult somatic tissues. If this is the case, it would be difficult to accept at face value claims that the observed protective effect was attributable to specific immunogenicity against tumor stem cells. Second, the authors propose that the particularly primitive nature of human ES cells (given their embryonic origin) could form the basis for the specific effects against CT26. Although this may be partially true, for the most part the data presented do not directly address this. Instead, it seems that aberrant (and indeterminate) exposure to human antigens, regardless of the origin of the cell line, was critically responsible for the observed effects. Although they will no doubt be laborious, experimental strategies aimed at discerning the immunospecificity of the effects of human ES cells will be necessary to resolve this question. Finally, the apparent difference between iPS and ES cells clearly deserves attention; however, future studies must address this variability as more than a mere formality. The evidence that human ES and iPS cells are different in any substantial way is, at best, preliminary. Until the authors perform the requisite well-controlled experiments, which are not trivial, it would be easy to mistake inter–cell line variations for intrinsic biological differences.

There is a certain irony in the fact that human ES cells, which themselves possess many features of neoplastic cells[6–8]—including sustained telomerase activity, formation of tumors after injection into mice, and infinite growth—would be exploited against cancer. By analogy, it is like fighting fire with fire. However, given the great uncertainty surrounding the nature of human ES cells, and thus the parameters that confer the ability to interact with the immune system, one must be cautious lest the positive analogy be replaced by the image of an 8-year-old boy playing with matches.

It is an obligatory exercise to dissect the specific effects of pluripotent cells and to determine how beneficial and safe they are relative to existing immunotherapeutic concepts. On balance, it is rewarding to see the potential spectrum of the application of human pluripotent stem cells growing far beyond what most of us have envisioned. This and other studies clearly indicate that the field of ES and iPS cells has entered a new, more mature era. As in most endeavors, timing is critical. But it is rewarding to see that the political climate with respect to science in general, and ES cells in particular, seems to be moving in a more favorable direction worldwide, especially in the United States. Scientific discoveries as well as progress on the political–ethical front will guarantee that stem cell research will have a profound and positive impact on the future of biomedical research and therapy.

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In the Beginning: Reflections on the Genesis of *Molecular Therapy*

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On the occasion of the tenth anniversary of the inaugural issue of *Molecular Therapy*, I would like to share my recollections of the events that led to the creation of the Journal, which occurred under my watch as the second President of the American Society of Gene Therapy.

It all began with a series of phone calls from our colleague, George Stamatoyannopoulos, in the summer of 1996 to several of us working in the field of gene therapy. George proposed to get together to talk about creating a new professional society focused on gene therapy. He summoned us to a meeting at the Marriott Hotel in San Francisco, CA, on 29 October

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