Molecular Therapy
Commentary

E pluribus unum: Combining Molecular Strategies to Defeat Head and Neck Cancer

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Head and neck squamous cell carcinoma (HNSCC) is one of the most treatment-resistant human malignant diseases. The disease is all too frequently impervious to the classic triad of surgery, radiotherapy, and chemotherapy. Better treatment for this miserable disease remains an unmet need, but the cancer is largely resistant to newer immunotherapeutic approaches with antibodies and immune cells.[1] In this issue of *Molecular* Therapy, using mouse xenograft models of the human tumor, Shaw and colleagues[2] first demonstrated partial efficacy of several molecular and immunotherapeutics in controlling tumor growth. They then combined these diverse approaches into a single successful treatment strategy that promises to represent a major improvement in the management of this lethal cancer.

Oncolytic adenoviruses that specifically target and destroy cancer cells show promise as a new approach to cancer treatment, but their efficacy is limited by the need to inject the virus directly into the tumor.[3] The Baylor group has used an imaginative approach that involves a series of genetic modifications to combine oncoviral tumor lysis with immunotherapy. They first targeted the tumor with a construct of two adenoviruses, one oncolytic and one helper-dependent, loaded with genes for anti-PDL1 (to inhibit checkpoint blockade)[4] and interleukin-12 (IL-12) (to drive cytotoxic T cells).[4] This novel viral construct, together with inflammatory signals from the injured and dying tumor cells, facilitated killing of primary and metastatic tumor by subsequent infusions of T cells genetically modified with a chimeric antigen receptor targeting the HER-2 tumor antigen on the HNSCC cells (Her2.CAR T cells). This extensive series of experiments can be summarized in two themes—first, the generation and enhancement of HER2.CAR T cells and, second, the use and modification of intratumorally injected adenovirus to promote persistence of HER2.CAR T cells at the tumor site.

Resistance to standard treatment in HNSCC is associated with overexpression of Erb family receptors, which include the tumor antigen HER2.[5] The group therefore selected HER2 as an attractive target for immunotherapy of HNSCC. However, while the HER2.CAR T cells they manufactured killed HER2-positive cell lines, there was minimal antitumor effect against established tumors in a xenogeneic mouse model.

CAR T cells can be rendered more cytotoxic by genetic modification to coexpress inflammatory cytokines, but this can lead to potentially lethal cytokine release effects. To more safely enhance the potency of the CAR cells, Shaw and colleagues[2] therefore explored the option of introducing cytokines into the tumor microenvironment. This was achieved by infecting cell lines or directly injecting tumors in the mouse with helper-dependent adenovirus (HDAd) constructs expressing one of 5 promising cytokines. Only the construct HDAd*IL-12p70* exerted an antitumor effect both in vitro and in vivo, confirming the potency of this cytokine in driving cytotoxic T cells.[6] However, the antitumor impact of combining the HDAd with HER2.CAR T cells was only modestly superior to that of HER2.CAR T alone. To further enhance the cytotoxicity of the PD1-expressing CAR T cells against the tumor, they next explored the impact of blocking PDL1 expression by the tumor so as to prevent T cell exhaustion through PDL1/PD1 engagement by CAR T cells at the tumor site.[7] After confirming that PDL1-blocking antibody enhanced HER2.CAR T cell killing of tumor lines, they made a further HDAd construct that included anti-PDL1. When coinfected with HDAd*IL-12p70*, this new construct (HDAd*PDL1*) significantly improved antitumor efficacy and survival in two HNSCC xenograft models (FaDu and SCC47). Among the various cytokines studied, only the IL-12 construct was effective. These animals showed persistence of the HER2.CAR T cells at the tumor site for up to 3 weeks together with an arrest of tumor growth. Subsequently, they created a single adenoviral construct expressing anti-PDL1 and IL-12 (HDAd*12-PDL1*) and showed that therapeutic efficacy was maintained.

While these stepwise modifications represented a significant improvement in the ability of CAR T cells to control the tumor, the investigators had reached the limits of optimization of the helper adenovirus approach. One drawback is that helper-derived adenovirus is unable to replicate in the tumor and cause oncolysis. Lytic damage to the cancer can only be achieved with an oncolytic adenovirus. Disappointingly, the oncolytic adenovirus (Onc.Ad) had minimal efficacy against both FaDu and SCC-47 tumors. However, the best results were achieved when they combined Onc.Ad with HDAd*12-PDL1* prior to CAR T cell treatment. In these animals, survival exceeded 100 days (compared with around 3 weeks for other variations). Furthermore, CAR T cells persisted at the tumor site and maintained their HER2 expression. The conclusion that all of the elements (Onc.Ad, HDAd*12-PDL1*, and HER2.CAR T) were necessary and sufficient for the winning combination was confirmed in large multicombinatorial experiments.

Promising results in primary tumor models do not immediately imply that treatments will have similar clinical efficacy. The

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investigators had to address the fact that more than half of patients with HNSCC have metastatic spread of the cancer in the lymphatic system.[7] How would their combined oncovirus CAR T cell approach work in a model closer to the clinical situation? To explore this, they set out to evaluate whether adenovival treatment at the primary site could enhance the impact of HER2.CAR T on distant metastases. They used a model where the FaDu tumor line metastasizes when injected into the tongue. They showed that, following CAd*12-PDL1* injection at the local site, the HER2.CAR T cells infiltrated both the primary and metastatic cancer sites. By fluorescently labeling the cancer cell line FaDu, they were able to show that the combinatorial treatment alone (CAd*12-PDL1* + HER2.CAR T) was capable of reducing tumor size and prolonging survival from 13 days to beyond 100 days, with persistence of HER2.CAR T at tumor sites for up to 120 days.

This logically planned and stepwise assembly of therapeutic components to achieve control of HNSCC represents a tour de force in gene modification of cells and viruses. The team is to be congratulated for the intuitive approach toward resolving, at least in preclinical models, the challenging clinical therapeutic problem of treatment-resistant head and neck cancer. However, this work is only the beginning of a long and difficult route to translation into workable therapy for patients with HNSCC. First, the strategy will invite strong regulatory scrutiny, involving not one, but two viruses, with one of them being gene modified as well as genetically modified CAR T cells. Second, there will be technical challenges in creating good manufacturing practices (GMP)-grade cellular and virus products for early-phase trials.[8] The patients selected for the first treatments in phase I studies will have advanced disease and will require skilled and possible intensive supportive care. While safety should be relatively easy to establish, efficacy will be hard to evaluate in patients with heavy disease burdens. Nevertheless, in the absence of any simpler strategies, the combination of oncolytic viruses with immunotherapy is definitely to be explored and should also be applied to other metastatic cancers, such as melanoma and pancreatic cancer.

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Targeting Cholesterol in Non-ischemic Heart Failure: A Role for LDLR Gene Therapy?

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The relationship between plasma cholesterol levels and non-ischemic heart failure has been controversial. In this issue of *Molecular Therapy*, Muthuramu et al. [1] address this issue by modulating plasma lipid levels by gene transfer of low-density lipoprotein receptor using an AAV8 vector (AAV8-LDLr) in a mouse model of cardiac pressure overload induced by transverse aortic constriction (TAC). AAV8-LDLr gene transfer induced a strong reduction of plasma cholesterol and lipoprotein levels, which then resulted in the attenuation of cardiac

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