changes in global methylation. This is reflected in the lack of changes in the differentiation pattern of CNS stem cells and failure of these mice to show evidence of glioma formation even with age.

These mouse models serve as an important step in modeling oncogenic *IDH* alleles and will undoubtedly serve as the basis for studies combining oncogenic *IDH* alleles with other mutations known to co-occur with *IDH1*/*IDH2* mutations in AML, glioma, and other malignancies. These results also raise fundamental biochemical questions as to whether specific enzymes are differentially inhibited by 2HG in a tissue-specific manner, and which of the many enzymes inhibited by 2HG are essential for transformation. In addition, further work is needed to bring greater resolution as to how epigenetic repatterning at key genes contributes to oncogenic transformation. Taken together, these studies demonstrate that *IDH1*/*IDH2* mutations will have pleiotropic effects in different contexts that contribute to transformation in different tumor types. Moreover, the development of these models provides an avenue for preclinical testing of IDH1/IDH2 inhibitors, such that we learn whether this represents a potential therapy for the subset of patients with neomorphic *IDH* disease alleles.

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# Tailor-Made Renal Cell Carcinoma Vaccines

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**Cancer vaccines are beginning to show signs of clinical activity, but major uncertainties remain regarding antigen selection, strategy for immune stimulation, patient stratification, and monitoring of elicited response. A new study of peptide vaccines in advanced renal cell carcinoma patients provides important insights into these central issues.**

Recent clinical successes have validated the long-standing idea that therapeutic manipulation of endogenous immunity may achieve meaningful anti-tumor effects (Mellman et al., 2011). Perhaps the most compelling evidence marshaled to date derive from studies of blocking monoclonal antibodies against key negative immune regulatory molecules, such as cytotoxic T lymphocyte-associated antigen-4 and programmed death-1, which achieve durable tumor regressions and/or disease control in several types of malignancies. These therapeutic approaches are limited in selectivity for amplifying anti-tumor immune responses, however, and thereby sometimes provoke serious inflammatory reactions in normal tissues.

Compared to targeting immune regulatory pathways, cancer vaccines offer a stronger potential for focusing immune reactions toward tumor-specific and tumor-associated antigens, but the clinical impact of these strategies thus far has been more modest, highlighting the need for further optimization (Mellman et al., 2011). Vaccines aim to load dendritic cells (DCs), the professional antigen presenting cells of the immune system, with relevant cancer antigens and stimulate DCs to mature and migrate to regional lymph nodes, where they may efficiently prime tumor antigen-specific T and B lymphocytes. The activated T cells, particularly cytotoxic $CD8^+$ lymphocytes but also $CD4^+$ effectors, may in turn traffic systemically to metastatic deposits and thereupon effectuate tumor

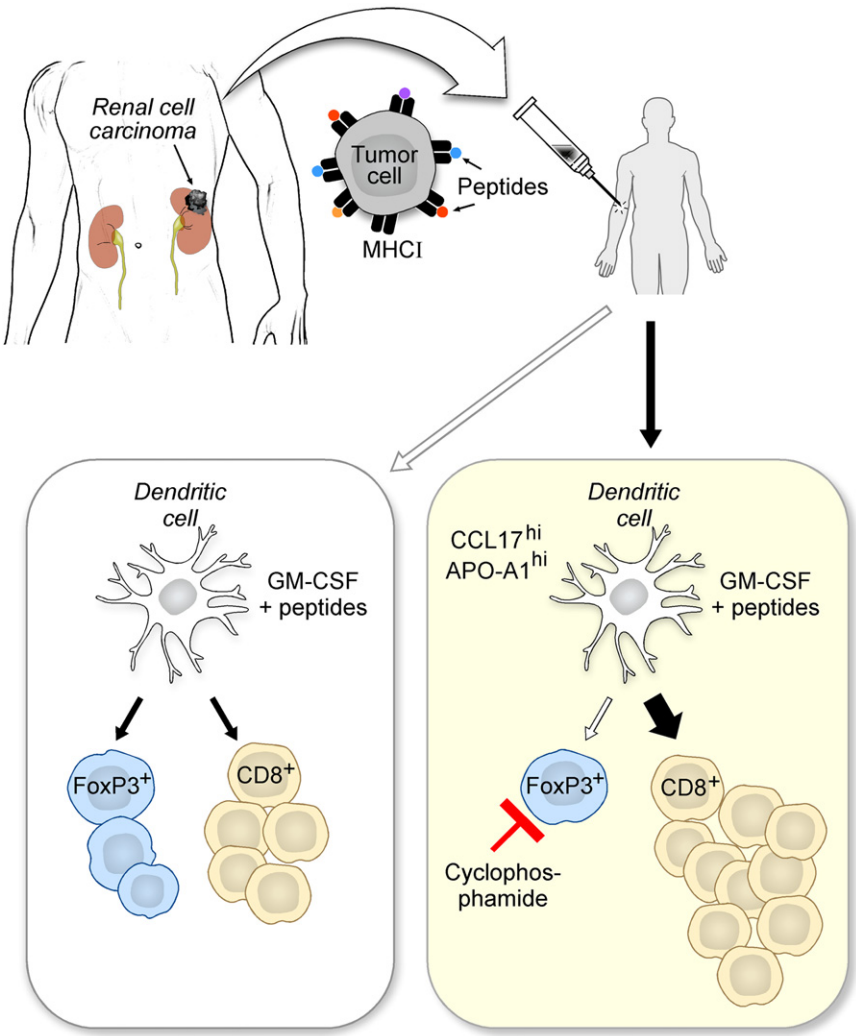


**Figure 1. Schema for Developing Tailor-Made Renal Cell Carcinoma Vaccines**
Naturally processed peptides derived from highly expressed oncogenic proteins are inoculated in conjunction with GM-CSF to stimulate tumor-specific $CD8^+$ cytotoxic T cells. The administration of cyclophosphamide prior to vaccination attenuates $FoxP3^+$ Tregs, resulting in more effective and durable anti-tumor immunity. High levels of apolipoprotein-A1 (APO-A1) and CCL17 are associated with superior vaccine outcome.

cell killing. Stimulated B cells may also differentiate into bone marrow-homing plasma cells that produce circulating anti-tumor antibodies.

While the general outline for a cancer vaccine may be reasonably crafted, many important details have yet to be clarified. In this context, the elegant study of renal cell carcinoma (RCC) vaccines by Walter et al. (2012) provides valuable insights into the choice of tumor antigen, method of immune priming, patient selection, and immune monitoring.

To identify relevant RCC antigens, the authors developed a multi-pronged strategy that combined biochemical, genetic, and immunologic techniques. Because the targets for $CD8^+$ cytotoxic T cells are short peptides (generated from any cellular protein) presented in the context of surface major histocompatibility complex (MHC) class I molecules, the authors purified these complexes from a large number of RCC samples and characterized the associated peptides with mass spectrometry. The expression profiles of the identified antigens were then determined, and those gene products showing the highest levels in RCC compared to normal kidney and other healthy tissues were prioritized. Lastly, the immunogenic potential of the peptides was interrogated using in vitro T cell stimulation assays with peripheral blood mononuclear cells obtained from healthy donors and RCC patients.

From this integrated analysis, the authors selected nine MHC class I peptides and one MHC class II peptide (for $CD4^+$ T cells) to serve as the antigenic components of the vaccine. The targets included sequences from the hepatocyte growth factor receptor tyrosine kinase MET, matrix metalloproteinase 7, cyclin D1, MUC-1, and two pro-angiogenic signaling molecules, all of which likely contribute to RCC progression. The choice of multiple, naturally processed peptides derived from proteins with critical oncogenic functions might reduce the emergence of antigen-loss tumor variants under immune selection.

To engender DC responses against these peptides, Walter and colleagues co-injected the cytokine granulocyte-macrophage colony stimulating factor (GM-CSF) that has been effectively employed in several earlier vaccine strategies, including Sipuleucel-T, the first FDA approved therapeutic cancer vaccine (Kantoff et al., 2010) (Figure 1). Consistent with the immunostimulatory properties of the vaccine formulation, the investigators observed that 20 of 27 evaluable patients with advanced RCC generated peptide-specific T cells in the blood that were detected with flow cytometry using MHC class I/peptide tetramers and functional assays for interferon-gamma production. Patients who mounted responses to multiple peptides showed longer survival compared to those with less intense or no responses, albeit the trial was not designed to assess clinical efficacy.

Additional immune analysis revealed that the induction of $CD8^+$ T cells to multiple peptide antigens was associated with lower numbers of FoxP3 expressing regulatory T cells (Tregs) at the time of study entry. This finding is consistent with other data indicating that Tregs negatively regulate the generation of anti-tumor cytotoxic T cells, likely reflecting their central role in maintaining immune tolerance (Josefowicz et al., 2012). Furthermore, recent work has also established the ability of GM-CSF to stimulate not only $CD8^+$ T cells, but Tregs as well, a feature that might limit vaccine potency (Jinushi et al., 2007).

Given these considerations, Walter and colleagues elected to administer a single dose of cyclophosphamide prior to the peptides/GM-CSF inoculation in an effort to attenuate Treg responses. Earlier work in murine models and small clinical trials illustrated that low doses of cyclophosphamide, which do not mediate a direct anti-tumor effect, might decrease Treg numbers (Le and Jaffee, 2012). To examine this possibility rigorously, the authors performed a Phase II trial in 68 advanced RCC patients who were randomized 1:1 to receive either the vaccine alone or a dose of cyclophosphamide prior to the first vaccine (a total of 17 vaccines were administered over several months to both groups). Indeed, cyclophosphamide reduced the numbers of circulating Tregs by approximately 20%, and this decrease was primarily in the subset of proliferating Tregs. In contrast, cyclophosphamide did not impact other lymphocyte populations analyzed, and patients who received only the vaccine did not manifest changes in Treg numbers.

Cyclophosphamide did not alter the frequency of patients who generated peptide-specific $CD8^+$ T cells, but the immune responders in this group achieved longer survival compared to immune responders who did not receive cyclophosphamide. Patients who failed to mount T cell responses displayed comparable survival regardless of cyclophosphamide treatment. Although the number of patients studied was relatively small, these results suggest that cyclophosphamide may modulate particular characteristics of vaccine responses that lead to more durable disease control. In accordance with this idea, a recent clinical trial in breast cancer that employed anti-CD25 blocking monoclonal antibodies to decrease Tregs in combination with peptide vaccines and GM-CSF similarly revealed augmented T cell responses and survival compared to earlier studies of vaccines alone (Rech et al., 2012).

To learn more about the factors that influence vaccine outcome, Walter and colleagues performed detailed immune profiling of patients prior to treatment. Among several cell types implicated in immune suppression (Gabrilovich et al., 2012), the authors found that increases in two myeloid cell populations ($CD14^+$ $HLA\text{-}DR^{-/lo}$ and $CD11b^+CD14^-CD15^+$) were associated with inferior overall survival. Moreover, among 300 soluble factors measured in the sera, high levels of apolipoprotein A1, a major constituent of high-density lipoprotein complexes, and the chemokine CCL17 were predictive for prolonged survival after vaccination. While the precise role of these proteins in immunization requires further study, one attractive possibility focuses on natural killer T (NKT) cells, a small population of lymphocytes that functions at the interface of innate and adaptive tumor immunity. NKT cells recognize lipid antigens and may activate DCs to stimulate $CD8^+$ cytotoxic T cells via a mechanism that involves CCL17 (Semmling et al., 2010).

Based on these two informative clinical trials, Walter and colleagues have launched a large Phase III trial to delineate the clinical efficacy of the peptide/GM-CSF/cyclophosphamide vaccine (IMA901) administered as a component of initial therapy for advanced RCC (http://clinicaltrials.gov number NCT01265901). Patients are randomized to sunitinib alone (the standard of care) or sunitinib plus IMA901, with overall survival as the primary endpoint. Prior work has suggested that sunitinib might be immunostimulatory, perhaps through diminishing Treg and myeloid suppressive cell numbers via inhibition of vascular endothelial growth factor receptor signaling (Gabrilovich et al., 2012). This integration of vaccines and targeted therapy represents a very promising approach to cancer treatment, and is likely to be soon followed with the addition of immunomodulatory antibodies (Vanneman and Dranoff, 2012). Overall, the careful clinical investigations of Walter and associates have helped to provide a strong framework for exploring innovative combinatorial therapies.

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