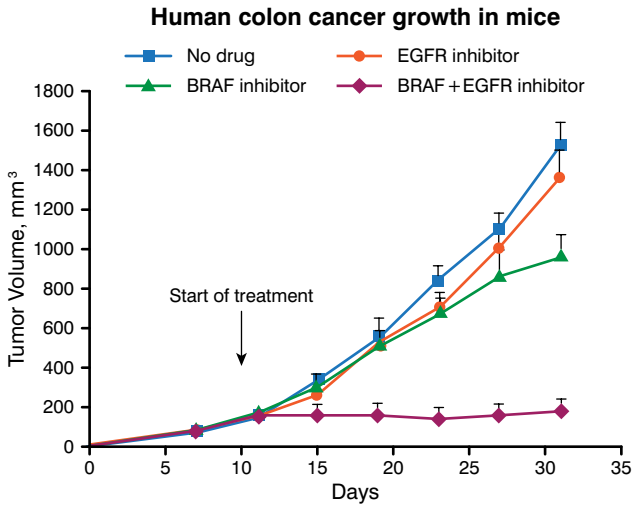
Figure 1. Suppression of Colon Cancer Growth by Simultaneously Inhibiting EGFR and BRAF



protein tyrosine phosphatases. The N-SH2 domain selectively binds to phosphotyrosyl motifs on receptor tyrosine kinases (RTKs), and it is required for activation of RAS signaling downstream of RTKs. BRAF-mutant colorectal cancer cells that lack PTPN11 are sensitive to vemurafenib. Knocking out PTPN11 also confers sensitivity to MEK inhibition in KRAS-mutant tumors.

In conclusion, the data show that many feedback systems are in play to reactivate cell surface receptors when a single pathway is inhibited. EGFR, KIT, c-MET receptors, and ERBB3 use PTPN11 for signaling downstream, and removing PTPN11 can alter feedback signaling. Inhibition of PTPN11, therefore, is a promising target for treating any cancer that suffers from RTK reactivation after primary therapy.

Precision Immunotherapy of Cancer

Written by Brian Hoyle

The traditional cancer therapy triumvirate of chemotherapy, radiation, and surgery is today being augmented with vaccines, cytokines, antibodies, targeted small-molecule drugs, and cell-based therapies such as immunotherapy. The hope is that combination therapies involving immune-based approaches will greatly enhance long-term survival.

Carl June, MD, University of Pennsylvania, Philadelphia, Pennsylvania, USA, offered an overview of genetically modified cancer therapy with T cells.

Cancer immunotherapy has its roots in the 1957 success of the first allogeneic bone marrow transplant and the hypothesis of immunosurveillance. Intervening research culminated most recently with the 2011 US Food and Drug Administration approval of ipilimumab for the treatment of unresectable or metastatic melanoma. More recent discoveries in the field include the benefits of inhibition of programmed cell death 1 (PD1) and programmed death-ligand 1 (PDL1) proteins in melanoma, non–small cell lung cancer, and renal-cell carcinoma; and the durable remissions produced in B-cell acute and chronic lymphoblastic leukemia using chimeric antigen receptor (CAR)-modified T cells.

The origin of T-cell immunosurveillance of tumors likely reaches far back in human evolution. From an evolutionary perspective, the immune system is better equipped for tumor tolerance than tumor elimination. Modern-day research seeks to build on our natural condition through synthetic biology by engineering T cells capable of tumor elimination. A number of synthetic-biology approaches under exploration to overcome tolerance of tumors include tumor-specific targeting by CAR-modified T cells and T cells with modified T-cell receptor (TCR) [Maus MV et al. *Blood.* 2014].

Tumor tolerance can be circumvented by engineering bi-specific T cells. This process involves either modification of the TCR domain or modification of T cells with an extracellular chimeric protein. The intent in both approaches is to target the T cells for a tumor type. The antibody-based CAR approach offers the advantages of "off-the-shelf" technology and independence from the major histocompatibility complex class.

In CAR technology, the chimeric construct that targets an antigen on the tumor cells (eg, CD19) is introduced into T cells, typically using a lentiviral vector. The resulting CART 19 cells that harbor the anti-CD19 CAR construct on their surface will bind specifically to the CD19 protein on tumor cells. The approach produces antigen-dependent killing of tumor cells [Milone MC et al. *Mol Ther.* 2009] with a T-cell population consisting of both effector (cytotoxic) and central memory T cells [Hollyman D et al. *J Immunother.* 2009]. Destruction of the tumor cells does not involve swelling [Kalos M et al. *Sci Transl Med.* 2011]. The engineered T cells kill tumors regardless of the tumor cells' response to chemotherapy. Thus, even chemotherapy-resistant tumor cells will be killed, as long as they express the target antigen on their surface.

Current data do not explicitly favor TCR over CAR or vice versa, Dr June noted. TCR requires only about 10 targets for functional performance, whereas CAR requires about 100 surface targets. The TCR requirement for major histocompatibility complex (MHC) 1 expression and human

leukocyte antigen (HLA) matching on the tumor cell, however, can be an important drawback that can permit tumor cells to escape immune surveillance. Both approaches provide long-lasting persistence of antitumor activity. It is likely that both will find their way into routine use, with a particular approach based on specific case circumstances.

Clinical trials of CAR T cells began in 1991, with the intent of retargeting T cells to treat patients with HIV. With more than a decade of use in patients, there is no evidence of integration near oncogenes or tumor suppressor genes, and no serious adverse events from the engineered T cells [Scholler J et al. *Sci Transl Med.* 2012]. Data indicate the safety of CAR-modified T cells as a therapeutic platform.

During the past 2 decades, refinements to the first-generation CAR T cells have improved the binding structure of the domain and the potency of the engineered T cells. Tumor tolerance is under the control of 4 types of CD4 T cells, all of which have different targets of activity (Th1, intracellular pathogens and cancer; Th2, extracellular pathogens; Th17, chronic inflammation and autoimmunity; and Treg, effector T cells). By tailoring the CAR design, the differentiation of Th1 and Th17 cell types can be programmed, which researchers feel will have implications for specific tumor destruction.

One recent therapeutic target for CAR T cells has been chronic lymphocytic leukemia (CLL). Individuals with fludarabine-refractory CLL have a median overall survival of only 10 months. A CAR designed to produce T cells expressing CD19 has been tested in a clinical trial of CLL patients [Grupp S et al. *N Engl J Med.* 2013]. To date, 3 late-stage CLL patients have responded to therapy, with reductions in cancer-cell numbers following CAR T-cell infusion.

Dr June and colleagues have also assessed the therapeutic value of CAR T cells in the treatment of pediatric and adult acute lymphoblastic leukemia (ALL). The team undertook a phase 1 trial [Maude SL et al. *N Engl J Med*. 2014] involving 30 patients (25 pediatric patients) with ALL, and they achieved a complete response in 90% of patients, with partial or no response in the remaining 10%, and a 6-month overall survival of 78% (95% CI, 64% to 95%).

The benefits of CD19 CAR T cells are accompanied by toxicities that include B-cell aplasia, tumor lysis syndrome, cytokine release syndrome, and macrophage activation syndrome. Nonetheless, these unprecedented survival results suggest that allogeneic transplantation for the treatment of ALL may eventually be replaced by CD19 CAR T-cell therapy.

Such compelling benefits have spurred developmental efforts toward leukemia and lymphoma targets, as well as other cancers, including glioblastoma, prostate cancer, pancreatic cancer, and lung cancer, at a variety of institutions worldwide.

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