provides a safe, yet potent cancer-specific immunotherapy for treating most epithelial malignancies and holds the potential to eradicate metastatic disease.

511. Engineered Donor Marrow Macrophages Phagocytose Cancer Cells and Aggressively Shrink Solid Tumor Xenografts Compared to Tumor Associated Macrophages

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Cell-based immunotherapies such as those based on engineered T-cells appear safe and often effective against liquid tumors. In solid tumors, macrophages are typically abundant, but the density of tumor associated macrophages (TAMs) correlates with poor clinical outcomes as they promote tumor growth, immunosuppression, and are nonphagocytic. In our studies, less differentiated donor marrow phagocytes are engineered to target tumors and selectively phagocytose cancer cells. Xenograft tumors were made on the flanks of NSG mice using a tdTomato human lung carcinoma cell line (A549). Systemic injections of anti-human IgG (anti-hum) with large tumors (~70 mm^2) showed no effect on tumor growth. However, systemic injection of bone marrow from donor NSG mice together with biweekly anti-hum treatments effectively stopped growth of the solid tumors. Replacing anti-hum with a non-specific antibody had no effect on tumor growth. Based on tdTomato signal intensity within macrophages isolated from tumors, 10-fold more donor macrophages are phagocytic compared to resident TAMs (2-3% are phagocytic). Since cancer cells express on their surface 'self' markers that limit the phagocytosis of these cells, we inhibited the 'self' receptors on the injected donor phagocytes prior to systemic injection of the donor marrow. This combination of 'self'-receptor inhibition with anti-hum causes a rapid decrease in tumor burden, shrinking tumors by ~40% in just 10 days compared to a similar growth of untreated tumors in the same time period. The anti-hum injection was again necessary as injection of a non-specific antibody failed to affect tumor growth. Tumor analysis showed that >85% of macrophages that were 'self'-receptor inhibited had phagocytosed the tdTomato A549 cells, which is ~30-fold greater than resident macrophages. Importantly, these cell therapy treatments appear safe with no significant decreases in hematocrit or platelets, which is unlike the anemia that has been reported upon systemic injection of 'self' inhibitors. Our results thus suggest that therapies based on engineered macrophages can be safe and effective against solid tumors if three requirements are met: a phagocytic phenotype, target opsonization, and inhibition of 'self' signaling.

512. The Cytokine Release Syndrome Crucially Contributes to the Anti-Leukemic Effects of CD44v6 CAR-T Cells

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Background: Despite the remarkable clinical results of CD19 CAR-T cells in B-cell leukemias, their long-term efficacy is limited by the emergence of CD19-loss escape variants. Moreover, whether the cytokine release syndrome (CRS) is necessary for durable remissions is a matter of debate. Currently available xenograft models in NSG mice are not suited for studying the antitumor effects of CAR-T cells beyond 3-4 weeks, because of xenograft-versus-host disease (X-GVHD). Moreover, since NSG mice lack functional myeloid cells, the CRS does not develop.**Aim**: To verify whether the CRS contributes to the antileukemic effects of CAR in an innovative xenotolerant mouse model.**Results**: NSG mice triple transgenic for human IL-3, GM-CSF and SCF (NSG-3GS) were sub-lethally irradiated and injected intra-liver with human HSCs soon after birth, enabling an accelerated and better balanced lympho-hematopoietic reconstitution compared with NSG mice. Reconstituting human T cells were single CD4+/CD8+ T cells, representing all memory sub-populations. After ex vivo isolation and activation with CD3/CD28-beads and IL-7/IL-15, NSG-3GS T cells were transduced with a CD44v6 CAR, retaining an early-differentiated (stem-cell/central-memory) phenotype and full antitumor functionality against acute myeloid leukemia (AML). NSG-3GS-derived CD44v6 CAR T cells were subsequently infused in tumor-bearing secondary recipients previously humanized with autologous HSCs. CAR-T cells persisted in vivo for at least 6 months and mediated durable leukemia remissions ($P<0.001$ vs controls) in the absence of X-GVHD. Tumor clearance associated with an acute malaise syndrome, characterized by high fevers and a surge in human IL-6 levels, which was lethal in 30% of the mice. Differently from CD19 CAR-T cells, the CRS by CD44v6 CAR-T cells was significantly anticipated (3 vs 8 days), coinciding with human CD44v6+ monocyte depletion. In humanized mice, previous myeloid-cell depletion by clodronate administration completely prevented this syndrome, but associated with late leukemia relapses. Conversely, mice developing the CRS entered a state of durable and profound remission, as demonstrated by prolonged observation times and secondary transplantation.**Conclusions**: By using an innovative xenotolerant mouse model, we have demonstrated that the CRS is needed for sustained antileukemic effects by CD44v6 CAR-T cells.

Cancer-Oncolytic DNA Viruses

513. Armed-Ad Gene Therapy Expressing PD-L1 Minibody Enhances the Anti-Tumor Effect of Adoptively Transferred Chimeric Antigen Receptor T-Cells for Solid Tumor Treatment

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Intratumoral treatment with oncolytic adenoviral vectors expressing an immunomodulatory molecule (Armed Onc.Ads) is safe and has shown some clinical benefit in patients with solid tumors. However, local treatment with Armed Onc.Ad has limited anti-tumor effect against metastasized tumors. T cells modified with tumor-directed chimeric antigen receptors (CARs) have shown promise for the systemic treatment of hematological malignancies, but have been less effective in treating solid tumors. Major reasons for this failure include lack of T-cell migration into solid tumors and the inhibitory microenvironments (e.g. PD-L1) at the tumor site. Recent clinical trials with immune-checkpoint inhibitors (e.g. anti-PD-L1 antibody) have broadly enhanced antitumor immunity by improving tumor-specific T cell responses. We therefore hypothesized that an Armed Onc.Ad expressing anti-PD-L1 antibody could enable the blockade of PD-1:PD-L1 interaction between CAR T-cells and cancer cells at the tumor site, and that combining these treatment modalities may have potent and synergistic anti-tumor effect in solid tumors. In this study, we confirmed that PD-L1 is upregulated on squamous cell carcinoma (Pre: 20%, Post: 98%), and prostate cancer cells (Pre: 60%, Post: 100%), in the presence of co-cultured IFNγ producing HER2.CAR T-cells, a population of effectors that we have safely