against CD5-negative cell lines. Upon longer-term coculture, CD5 CAR T cells eliminated >95% of leukemia cells from 3 T-ALL lines within 48h and 100% by day 7. We also observed the ability of CD5 CAR T cells to eliminate leukemia cells in sequential killing assays where we recurrently replenished fresh target cells for at least 4 iterations. Lack of functional exhaustion in sequential killing assays supports the fitness of CD5 CAR T cells for eradicating large numbers of tumor cells in vivo. CD5 CAR T cells dramatically suppressed systemic in vivo disease progression in 3 different xenograft mouse models, doubling median survival. Importantly, CD5 CAR T cells demonstrated significant cytokine production and cytotoxicity against primary T-ALL blasts (n=6), highlighting the therapeutic potential of CD5 CAR for patients with T cell malignancies. Overall, we demonstrated for the first time that CD5 CAR redirects T cells to eliminate CD5-positive malignant T cells in vitro and in vivo while producing only limited fratricide of the normal T cell population.

412.   Development of GD2-Specific Immunoliposomes for Immunotherapy of Neuroblastoma

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Tumor growth creates a highly immunosuppressive tumor microenvironment (TME) that impairs T-cell localization, persistence, or the execution of their effector function. This represents a major and as yet unsolved critical challenge to the development of effective adoptive immunotherapy of solid tumors. To selectively target TME in neuroblastoma and make it permissible for survival and function of tumor-specific T cells, we have developed a novel nanoparticle (NP) delivery platform which consists of 150 nm immunoliposomes rendered specific for neuroblastoma cells using the Fab fragment obtained from the anti-GD2 mAb clone 14g2a. To ensure high density surface coverage and correct orientation of anti-GD2 Fab on the NPs, we synthesized a fusion protein consisting of 14g2a Fab and folate receptor (Fab14g2a-FR) and attached it on the outer layer of the immunoliposomes enriched with folic acid. Rhodamine-labeled GD2-specific but not control NPs could specifically bind GD2-positive CHLA-255 neuroblastoma cells but not GD2-negative LA-N-6 neuroblastoma cells in vitro as determined by FACS. To examine the in vivo biodistribution of NPs, DiR-labeled GD2-specific or non-specific NPs were injected to NOD/SCID mice implanted with CHLA-255 cells. Tumor tissues and normal organs were imaged after 72 hours using ex vivo fluorescence imaging. Up to 58% of GD2-specific NPs accumulated at the tumor sites. The only other organ with significant accumulation of NPs was the liver. Minor traceable portions were detected in the spleen and lung. To utilize the observed targeting capabilities of GD2-specific NPs to achieve antitumor effects, we are now loading NPs with recombinant human (rhIL-7) and will test whether the preferential delivery of rhIL-7 to the tumor site and liver, a major site of NB metastasis, will increase the survival and anti-tumor activity of T and NKT cells engineered to express a GD2-specific chimeric antigen receptor with IL-7Rα. The results of this study will inform design of immunotherapy of neuroblastoma and other tumors in combination with TME-modifying NPs.

413.   Pre-Clinical Preparation and Validation of Tumor Cell-Based IL-12 Immunotherapy for Acute Myeloid Leukemia

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Interleukin(IL)-12 is a potent pro-inflammatory cytokine that stimulates a variety of effector cells involved in anti-tumor immunity. Systemic administration of IL-12 has associated toxicities, however. Various strategies are being developed to reduce such toxicities by restricting IL-12 distribution. Options here include generating fusions with tumor-targeting molecules and directing gene delivery to specific cells. First - we used lentivirus vectors (LVs) to engineer expression of murine IL-12 in tumor cells ex vivo and subsequently infused such modified cells into recipient mice. This strategy restricts IL-12 to the local tumor microenvironment thereby promoting immune activation in the context of tumor-associated antigens (TAAs). Using mouse models of both leukemia and solid tumors, we found that this cell-based approach generated effective anti-tumor protection when as little as 1% of the tumor burden expressed IL-12 as long as threshold expression levels on a per cell basis were reached. Second - our groups showed that anti-tumor mechanisms here involve CD4+ killer T cells, dendritic cells, and direct cell-cell contact with effectors. Clinical translation of this cell-based IL-12 therapy is in progress in Toronto for AML using a novel LV to modify patients' own blast cells. Patient AML cells collected to date (n = 21) were stratified based on in vivo growth kinetics and transduced with a near-GMP-grade bicistronic LV that encodes the human IL-12 cDNA as a p40-p70 fusion, as well as a mutant thymidylate kinase (tmpK) fused to the ectodomain of LNGFR (trLNGFR) as a suicide (cell-fate control) cassette. The trLNGFR/tmpK element allows selection and also selective ablation of transduced cells by administration of AZT. Furthermore, it also allows tracking of transduced cells and quantification of transduction frequencies/transgene expression levels. With our current protocol, functional transduction efficiencies of primary patient AML blasts ranged from 20% to 70% (n=17). Transduced AML cells displayed a strong correlation between vector copy number and trLNGFR/tmpK + IL-12 levels and dose-dependent sensitivity to AZT. In vitro immortalization (IVIM) assays determined that the near-GMP LV/IL-12 vector displayed minimal genotoxic risk in transduced lin- cells; insertion site analyses carried out on expanded clones displayed poly- to oligo-clonality patterns. Pre-clinical data on toxicity and scale-up considerations are being accumulated in preparation for a Clinical Trial Application to Health Canada targeting AML. This LV/IL-12 immunotherapy platform targeting tumor cells themselves thus holds potential to be effective against a wide variety of cancers.

414.   CAR Spacers Including NGFR Domains Allow Efficient T-Cell Tracking and Mediate Superior Antitumor Effects

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Introduction. Chimeric antigen receptors (CARs) frequently include an IgG1-CH2CH3 spacer conferring optimal flexibility for antigen engagement and allowing the selection and tracking of CAR-expressing T cells. A serious drawback of CH2CH3-spaced CARs

is however their interaction with Fcγ receptors (FcgRs). Indeed, this antigen-independent binding may lead to the unintended elimination of cells expressing these receptors (mainly phagocytes), foster the development of non-specific immune reactions and drastically decrease the efficacy of the strategy due to the premature clearance of transduced T cells in vivo.

Material and Methods. We designed and constructed novel CAR backbones by substituting the IgG1-CH2CH3 spacer with regions from the extracellular portion of the low-affinity nerve-growth-factor receptor (LNGFR), differing for the length and potential binding to NGF. In particular, we used our recently developed CD44v6-specific CAR as a model for comparing the antitumor activity of the different LNGFR-based designs both in vitro and in vivo.

Results. After transduction, all constructs could be identified on the T-cell surface using anti-LNGFR antibodies, indicating that they were correctly processed, mounted on the cell membrane and still recognized by anti-NGFR antibodies. As a consequence, all the LNGFR-based spacers allowed selecting CAR-T cells with immune-magnetic beads coupled to anti-NGFR antibodies, without interfering with their expansion and functional differentiation after activation with CD3/CD28 beads plus IL-7 and IL-15. Most importantly, LNGFR-spaced CAR-T cells maintained potent cytotoxic, proliferative and cytokine-release activity in response to CD44v6-expressing leukemia and myleoma cells, while lacking antigen-independent recognition through the FcgR. Noticeably, even at supra-physiological NGF concentrations, the LNGFR-spaced CD44v6-CAR.28z CAR T cells were not induced to proliferate, indicating the absence of signaling via soluble NGF. Strikingly, LNGFR-spaced CAR-T cells better expanded and persisted in vivo compared to CH2CH3-spaced CAR-T cells and mediated superior antitumor effects in a well-established tumor disease model. Interestingly, we demonstrated that the premature disappearance of CH2CH3-spaced CAR-T cells was due to engulfment by murine phagocytes, a phenomenon not occurring with LNGFR-spaced T cells.

Discussion: In conclusion, we demonstrated that the incorporation of the LNGFR marker gene directly in the CAR sequence allows for a single molecule to work as a therapeutic and as a selection/tracking gene and shows an increased efficacy/safety profile compared to the IgG1-CH2CH3 spacer.

415. First-In-Patient Proof of Safety and Efficacy of a 4th Generation Chimeric Antigen Receptor-Modified T Cells for the Treatment of Relapsed or Refractory CD30 Positive Lymphomas

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Background: Many lymphoma patients cannot be cured by standard chemo-radiotherapy. CD30 is expressed in Hodgkin's lymphoma (HL), anaplastic large cell lymphoma (ALCL), diffuse large B cell lymphoma, and peripheral T/NK cell lymphoma. Brentuximab (SGN-35), an antibody-drug against CD30, has been approved by U.S. Food and Drug Administration (FDA) for the treatment of relapsed or refractory classical HL and systemic ALCL. However, SGN-35 is not available or approved in many countries. Nevertheless, CD30 represents an attractive target for chimeric antigen receptor (CAR)-based immune cell therapy. This study reports the safety

and efficacy of a 4th generation CAR T cell treatment for the management of relapsed and refractory CD30 positive lymphomas (www.clinicaltrials.gov; #NCT02274584).

Methods: Lymphoma patients with relapsed or progressive CD30 positive disease are recruited. T cells are transduced with lentiviral CAR containing anti-CD30-scFv and T cell signaling domains including CD28/CD137/CD27 and CD3zeta. The CAR is fused with an apoptosis-inducing gene, FKBP-caspase 9 (iCasp9), to establish a safety-improved CAR (4S-CAR). CAR T cells and cytokines in blood are detected by quantitative PCR and ELISA, respectively.

Results: A 22-year-old male, diagnosed with stage III HL (Nodular Sclerosis, NS) in December 2011, had been heavily treated with three lines of chemotherapy and auto-transplantation. The patient relapsed in May 2014 and has been enrolled in this study. He received a conditioning regimen of three daily doses of fludarabine 25mg/m^2 and cyclophosphamide 250mg/m^2 one week before CAR T infusion. The total cell number infused was 3.2x10^8, of which 5% were CAR-positive. There were no infusion-related toxicities. 35 days and 2.5 months after infusion, CT scan showed resolution of multiple tumor nodules, which indicated partial remission. However, 5 months after infusion, disease slowly progressed based on CT scan. The CAR T cells peaked on day 45 accounting for >20% of circulating lymphocytes. Peak levels of interferon-γ and interleukin-6 were detected around day 40 coincided with peak CAR T detection.

Conclusions: We demonstrate for the first time the safety and efficacy of CD30 4S-CAR T cells in a heavily-treated, relapsed late-stage CD30 positive HL patient. Compared with leukemia and other subtypes of lymphoma, HL has unique pathological characteristics. The NS subtype is the most common HL characterized by dense bands of collagen fibrosis and an overt immunosuppressive tumor niche. Such feature may result in the difficulty of CAR T cells to penetrate into the tumor mass. We are designing new treatment regimens to overcome this obstacle. Expansion of patient cohort and long term follow-up are in progress.

416. Immunotherapy of Hepatocellular Carcinoma With T Cells Engineered To Express Glypican-3-Specific Chimeric Antigen Receptors

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Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer-related death worldwide, with no curative therapies for unresectable HCC. Glypican-3 (GPC3), a membrane bound heparan sulfate proteoglycan is selectively expressed on HCC and has recently emerged as an attractive target for immunotherapy. GPC3-specific monoclonal antibody, GC33 has been shown to be safe in recent a Phase 1 clinical study. However, objective clinical responses to GC33 treatment were modest and transient. We hypothesized that the therapeutic efficacy of GPC3-targeting immunotherapy can be enhanced by combining the specificity of GC33 mAb with the advantages of adoptive cell therapy. To that end, we generated T cells genetically engineered to express GPC3 specific chimeric antigen receptors (GPC3 CARs).

The signaling parts of GPC3 CAR constructs contained CD3ζ chain only (1st generation), with CD28 or 4-1BB (2nd generation), or both (3d generation) costimulature endodomains. We found that GPC3 CAR T cells efficiently and specifically killed GPC3-positive HCC cell lines in vitro (figure 1); released IL-2 and IFN-γ, and proliferated in response to stimulation by HCC cell lines.