21. Non-CD3-Based Chimeric Antigen Receptor (CARs) with Enhanced Anti-Tumor Activity in Solid Tumors

Enxiu Wang,[1] Liang-Chuan Wang,[2] Ching-Yi Tsai,[1] Vijay Bhoj,[1] Steven Albelda,[2] Edmund Moon,[2] Michael Milone.[1]
[1]Department of Pathology & Laboratory Medicine, Perelman School of Medicine of the University of Pennsylvania, Philadelphia, PA; [2]Department of Medicine, Perelman School of Medicine of the University of Pennsylvania, Philadelphia, PA.

Chimeric antigen receptors (CARs) based upon a single chimeric molecule bearing an antigen binding domain linked in cis to the cytoplasmic domains of CD3ζ and costimulatory receptors CD28 or 4-1BB provide a potent method for engineering T cell cytotoxicity towards tumors. We describe a simple chimeric receptor based upon a killer immunoglobulin-like receptors (KIRs) normally expressed by natural killer (NK) cells and T cells. Constructed by fusing a single chain variable fragment (scFv) to the transmembrane and cytoplasmic domain of a KIR, we show that a KIR-based CAR targeting multiple tumor-related antigens triggers antigen-specific cytotoxic activity and cytokine production that is comparable to CD3ζ-based CARs with antigen-induced proliferation that is independent of additional costimulation. Using a solid tumor xenograft model that is highly resistant to CD3ζ-based CAR-targeted T cells, we further demonstrate that KIR-based CAR-targeted T cells can induce tumor regression despite in vivo persistence of CD3ζ-based CAR-modified T cells with CD28 and 4-1BB costimulatory domains. We show that the enhanced activity of the KIR-based CAR is associated with less tumor-induced T cell hypofunction that has previously been demonstrated with CD3ζ-based CARs. These data support the future clinical evaluation of a KIR-based CARs especially within solid tumors.

22. A Novel Single-Chain Antibody for Selective Targeting of IL13Rα2-Expressing Brain Tumors

Julius W. Kim,[1] Jacob S. Young,[1] Maciej S. Lesniak,[1] Irina V. Balyasnikova.[1]
[1]Surgery, The University of Chicago, Chicago, IL.

BACKGROUND: IL13Ra2 is overexpressed in majority of high grade astrocytomas and other malignancies, and has been validated as a target for therapeutic applications in various pre-clinical models. However, current IL13-based therapeutic agents lack specificity due to interaction with the IL13Ra1 receptor, which is widely expressed by normal cells. The generation of a targeting agent that strictly binds to IL13Ra2 would significantly expand the therapeutic potential for the treatment of IL13Ra2-expressing cancers. Recently, we developed and extensively characterized monoclonal antibody 47 (mAb47), which exclusively binds to a native form of human IL13Ra2. The goal of this study was to engineer a single-chain antibody (scFv) from the parental hybridoma cell line, test its targeting properties as a soluble agent, and create an adenovirus (Ad) with a modified fiber incorporating scFv47 as a targeting motif.

METHODS: The phage-display approach was utilized for selection of functional combination of variable heavy and light chains from established hybridoma cells producing mAb47. Purified phages displaying scFv47 were tested for their interaction with IL13Ra2hFc recombinant protein. A competitive ELISA was utilized to verify if parental mAb47 and scFv47 share the same epitope. The soluble form of scFv47 expressed in E. coli and CHO cells was analyzed by SDS-PAGE, and tested for stability and targeting properties. To generate IL13Ra2-specific Ad, the fiber of a replication-deficient Ad5 encoding green fluorescent protein was replaced with a chimeric fiber gene composed of a T4 fibritin trimerization domain linked at its C-terminal to scFV47 (AdFFscFv47-CMV-GFP). To generate viral particles, a construct encoding the adenoviral genome was rescued in HEK293F28 cells, propagated, and purified. IL13Ra2+ and IL13Ra2- U251 cell lines were established via stable transfection with either control or IL13Ra2-specific shRNAs (U251-IL13Ra2. KO), respectively. The AdFFscFv47-CMV-GFP virus was tested for targeting properties in these U251 cell lines and IL13Ra2-expressing U87 glioma cells.

RESULTS: The biopanning-selected pool of phages, as well several individual clones, demonstrated specific binding to IL13Ra2hFc protein, but not to hIgG in plate ELISA. Binding of scFv47-displayed phages to IL13Ra2 was completely abolished by the mAb47, but not control IgG or other tested IL13Ra2 mAbs, thus confirming the same IL13Ra2 epitope for scFv47 and parental mAb47. Similarly to phage-displayed scFv47, the soluble scFv47 showed specific binding to IL123Ra2, but not IL13Ra1. Interaction of Ad5FFscFv47-CMV-GFP was also specific to IL13Ra2-expressing U251 cells, as judged by flow cytometry for GFP expression in U251-IL13Ra2+ versus U251-IL13Ra2.KO cells. Furthermore, GFP expression in cells infected with Ad5FFscFv47-CMV-GFP strongly correlated with the level of surface expression of IL13Ra2. The specificity of viral infection was further validated in a U251 glioma model.

CONCLUSION: Our data validate the scFv47 as a highly selective IL13Ra2 targeting agent and open venues for the exploration of scFv47-based viral, cell and protein therapeutics for the treatment of various IL13Ra2-expressing human malignancies.

23. Uncovering Mechanisms of Resistance to Cetuximab by Insertional Mutagenesis in Heterotopically-Engrafted Human Colorectal Cancers

Valentina Pirazzoli,[1] Riccardo Biavasco,[1] Giulio Spinozzi,[1] Andrea Bertotti,[2] Livio Trusolino,[2] Eugenio Montini.[1]
[1]San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milan, Italy; [2]Laboratory of Translational Cancer Medicine, Candiolo Cancer Institute (FPO-IRCCS), Turin, Italy.

The development of high-throughput technologies has made possible the identification of cancer-driver mutations, which account for the growth and spreading of cancer cells. The discovery of these specific cancer biomarkers led to the development of anti-cancer targeted therapies, which hit in a specific manner cell pathways directly involved in tumor progression. This new class of therapeutic agents, which comprehend small molecules and antibodies, has been shown to be more effective and less toxic than conventional chemotherapy in advanced forms of cancer. However, the inevitable development of acquired resistance, due to the acquisition of multiple mutations or activation of compensatory pathways, has limited their success. A paradigm for this concept are the anti-Epidermal Growth Factor Receptor (EGFR) monoclonal antibodies, cetuximab and panitumumab, which are Federal Drug Administration (FDA)-approved agent for the treatment of EGFR-expressing metastatic colorectal cancer (mCRC). Unfortunately, despite the massive initial reduction, response is transient and tumors become refractory within 12-18 months.

We take advantage of a lentiviral vector (LV)- based insertional mutagenesis platform to induce cetuximab-resistance in Patient-Derived Xenografts (PDXs) of mCRC, with the final goal of uncovering the molecular mechanism of the resistance. Insertional mutagenesis has been successfully used in our lab to explore novel genes involved in the resistance to lapatinib in two breast cancer cell lines and to erlotinib in a pancreatic cancer cell line. Given these encouraging results we now aim to apply this technology to human cancer specimens to better reflect the real clinical response. Established PDXs from cetuximab-sensitive liver metastatic lesions of CRC have been excised, chemically digested to single cell suspension

and consequently transduced with a genotoxic LV, harboring the hyperactive enhancer/promoter of Spleen Focus Forming Virus in the Long Terminal Repeats (LV-SF-LTR) sequences or a non-genotoxic LV, containing self inactivating LTR sequences. Transduced tumor cells have been re-implanted in NSG mice to reconstitute the tumors. Once engrafted, mice have been divided in two groups receiving either cetuximab or the drug-vehicle. LV-SF-LTR transduction of a large amount of cells with a high vector copy number will allow us to randomly hit genes responsible for the resistance to the tumor-specific targeted therapy and deregulate their expression. Therefore, exposure to the treatment will not rest tumor growth in these tumors. LAM-PCR and deep sequencing analysis performed on drug-sensitive and drug-resistant tumors will be used to map the integration sites of the LVs and consequently to identify LV-induced gene deregulations responsible for the pharmacological resistance.

The identification of the biomarkers accounting for the drug-resistance is a real challenge that will allow screening for rational drug combinations to reverse resistance, improving the life expectancy of CRC patients.

24. Targeted Anticancer Approach Through microRNA-Regulated Tumor-Specific RNA Replacement

Ju-hyun Kim,[1] Jin-Sook Jeong,[2] Seong-Wook Lee.[1]
[1]Dept. of Molecular Biology, Institute of Nanosensor and Biotechnology, Research Institute of Advanced Omics, Dankook University, Yongin, Republic of Korea; [2]Dept.of Pathology and Medical Research Center for Cancer Molecular Therapy, Dong-A University College of Medicine, Busan, Republic of Korea.

We have previously presented that trans-splicing ribozymes based on Tetrahymena group I intron could be potent anti-cancer devices through diminishing target RNA level and simultaneously triggering anti-cancer gene activity only in cancer cells expressing the target RNA. Particularly, hTERT-targeting ribozyme was shown to specifically and efficiently retard tumors in various xenograft carcinomatosis nude mouse models. However, concerns for the hTERT-targeting approach include potential side effects to proliferating hTERT+ normal cells. In this study, to improve cancer-specificity of the ribozyme, we developed cancer-specific ribozyme expression construct via microRNA regulation. To circumvent hepatocellular carcinoma (HCC) as target models, we created tissue-specific microRNA-regulated ribozyme system by incorporating perfect antisense sequence against normal hepatocyte-selective miRNA-122a that is down-regulated in HCC to the 3'-UTR region of ribozyme. Adenovirus encoding the modified hTERT-targeting ribozyme specifically and efficiently induced transgene and cytotoxicity in liver cancers through cancer-specific RNA replacement. Moreover, specificity of cancer regression was highly increased without compromising its anti-cancer efficacy in xenograft orthotopic HCC model. Furthermore, to assess the effect of this system in syngeneic model, we constructed mTERT-targeting ribozyme harboring miRNA-122a target sites. Adenoviral vector encoding the modified ribozyme selectively and efficiently regressed mTERT+ HCC with minimal liver toxicity due to least ribozyme expression in the normal livers in syngeneic orthotopic mouse model. Noticeably, cytotoxicity caused by the adenovirus induced specific immunogenic cancer cell death in the syngeneic model, thereby completely suppressing secondary tumor challenge in the mouse. Therefore, specifically targeted transgene induction through microRNA-regulated RNA replacement provides novel strategies for efficient and safe cancer gene therapy.

Hematologic and Immunologic Diseases: Clinical Trials and Observations

25. A Dose Escalation Study of Cyclophophamide (CTX) to Enhance SB-728-T Engraftment

Dale Ando,[1] Gary Blick,[2] Jay Lalezari,[3] Ricky Hsu,[4] Edwin DeJesus,[5] Trevor Hawkins,[6] David Parks,[7] Shelley Wang,[1] Winson Tang,[1] Ron Mitsuyasu.[8]
[1]Sangamo BioSciences, Richmond, CA; [2]Circle Care, Norwalk, CT; [3]Quest, San Francisco, CA; [4]New york, New York; [5]Orlando Immunology Center, Orlando; [6]Southwest Care, Santa Fe; [7]Central West Clinical Research, St Louis; [8]UCLA, LA.

Background: CCR5 modified autologous CD4 cells (SB-728-T) are safe and increases total CD4 counts. The cells traffic to lymphoid tissues and have a selective survival advantage during ART treatment interruption (TI). Additional studies in CCR5 D32 heterozygote HIV subjects showed VL reductions during TI correlated with circulating bi-allelic CCR5-modified CD4 cells supporting the importance of maximizing engraftment. Low dose CTX has been successfully used to increase T cell engraftment. This study examines the effect of escalating doses of CTX on SB-728-T engraftment.

Methods: A dose escalation study of IV CTX, with doses ranging from 100 mg/m2 to 2 g/m2 (n=3-6/cohort), administered 1-3 days prior to SB-728-T (>90% CD4, <1% CD8) infusion was performed in 18 aviremic, ART treated HIV subjects with CD4 T cells ≥500/uL.

Results: CTX was generally well tolerated with low grade GI side-effects, managed with anti-emetics, at doses up to 1 g/m2. Grade 3 and 4 neutropenia requiring G-CSF developed at 1.5 and 2.0 g/m2 CTX. On Day 7, a dose-related increase in CD4 count and engraftment of bi-allelic CCR5 modified cells was observed with CTX doses up to 1 g/m2 but did not increase at 2.0 and 1.5 g/m2. By comparison, there was a progressive decline in CD8 cells with CTX dose escalation. Data is expressed as Mean +/- SE in Table 1.

Table 1

	100 mg/m2	500 mg/m2	1 g/m2	2 g/m2	1.5 g/m2
ΔCD4 (cells/uL)	776 ± 502	1695± 518	2700 ±966	1370 ± 721	1396 ± 367
ΔCD8 (cells/uL)	98 ± 49	180 ± 117	-210 ± 7	-424 ± 63	-164 ± 161
Bi-allelic (cells/uL)	55 ± 42	102 ± 24	169 ± 67	142 ± 30	180 ± 25

A 1-log VL reduction from peak was seen in 1 subject each at 100 and 500 mg/m2 of CTX while 1 subject each at the 1 and 1.5 g/m2 dose level had a 2-log decline during a TI. At the conclusion of the study, 3 additional subjects were conditioned with 1 g/m2 of CTX and administered CCR5 modified T cells containing 46.9+/-6.4% CD8 cells. CD8 count increased by 2236+/-967/uL (range 1029-4150/uL) with only modest increases in CD4 counts (733+/-233/uL; range 297-1096/uL) at 7 days in the 3 subjects. Two of the three subjects have had modest increases in VL to date during TI (<1000 copies/mL), suggesting an effect on viral control with the added CD8 T cells.

Conclusion: CTX conditioning is generally well tolerated and was associated with increased engraftment of CCR5-modified T cells at doses up to 1 g/m2 in HIV subjects. CTX conditioning may be a useful strategy to maximize the engraftment and anti-viral effects of SB-728-T. The effects of co-administering CD8 cells with SB-728-T on VL will be presented.