Genome Editing and Engineering

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Module - 10
Engineered immune cells for Cancer therapy (II)
Lecture - 03
Cancer therapy (II) -Part A

Welcome to my course on Genome Editing and Engineering. We are discussing about engineered immune cells for cancer therapy. This is the lecture number two of this particular topic. So, let us begin today's discussion with CART T-cell based adaptive immunotherapeutics. Cytotoxic T-cells play a key role in anti-tumor immunity and due to impaired recognition of CTLs to tumor cells lead to immune evasion. Hence, regaining the ability of targeted recognition is critical for targeted immunotherapy.

Tumor specific T cells that are naturally present in patients with malignancies are relatively low and their function is impaired which makes it difficult for T cell based adoptive transfer. In addition to neo antigen specific T cell receptors, genetically modified patient derived T cells bearing chimeric antigen receptors can be generated as therapeutic cellular products with a high tumor specificity. Chimeric antigen receptors are genetically modified receptors introduced and expressed in human T cells for targeting the surface antigens of tumor cells in their native conformation.

And this is done by conventional genetic engineering methods as well as the developing genome editing technologies. They can be subjected to ex vivo expansion and clinically administered via adaptive transfer to patients. CAR T cells is a chimera of binder domain typically derived from an antibody with T cell derived transmembrane and intracellular signaling domains. We will once again discuss this when we show the diagram for these CAR T cells. CARs harness the exquisite MHC-independent binding specificity of antibodies to activate T cells via TCR and co-stimulatory signaling domains.

Affinity tuning of binder domains and selection of signaling domains are important considerations in CAR-T cell therapy. High affinity binder domains prevent tumor escape

by antigen low cancer cells, whereas low affinity domains confer increased cytotoxicity and proliferation and can spare normal cells expressing physiological levels of target antigen. CARs bind their antigens directly and are not limited to the proteome, but expand to other macromolecules such as glycans, which can differ markedly between normal and tumor cells. However, unlike TCR T cells, only surface-accessible or secreted antigens can act as targets for CARs. This is a picture of a chimeric antigen receptor structure.

CARs are composed of a membrane-distal single-chain variable fragment (scFv), a spacer domain, a transmembrane domain and intracellular activation or signaling domain. In contrast to TCR, CARs recognize surface antigens in a non-MSC manner. TCR identifies intracellular or extracellular proteins that are presented as peptides by MSC molecules. CAR T-cells are typically produced by transducing T-lymphocytes with a transgene encoding a synthetic antigen receptor. This transgene is integrated into the T cell genome transcribed and translated into a CAR protein.

So, you can see this transgene and this is the CAR T-cell to which this gene is transferred and you can see the translated product on the right. The binding and signaling domains separated into extracellular and intracellular compartments respectively. The extracellular scFv domain is derived from the variable region of an antibody that recognizes specific tumor antigens together with a spacer that provides flexibility to the binding domain. The transmembrane domain connects the binding domain with intracellular signaling moieties. The TCR-derived CD3-zeta chain drives T-cell activation and is fused in tandem with co-stimulatory endodomains that allow for robust and sustained functions. The extracellular single chain variable fragments works for antibody like antigen recognition and intracellular signaling domains for activating T cells.

In CAR T-cells, the extracellular domain scFv is responsible for redirecting the specificity of CTLs to the malignant cells and can be designed according to specific antigens such as CD19 expressed in B-cell acute lymphocyte leukemia, chronic lymphocyte leukemia and lymphoma. CAR intracellular signaling domains provide the necessary signals for priming T-cell activation. In the CAR T-cell mediated immune response, the ScFv of CARs can engage surface antigens of tumors directly via

antibody-like binding. The heavy-chain variable region and light-chain variable region of antibodies are linked by a small segment of polypeptide.

The hinge domain is composed of immunoglobulin superfamily members such as CD8, CD28 or IgG, which plays a role in signal transduction. The intracellular signal transduction region is mainly composed of the CD3 zeta chain of the TCR. In addition to intracellular signaling domains, co-stimulatory molecules such as CD28 or 4-1BB can improve cell proliferation and survival time in vivo and enhance the anti-tumor activity of CAR T-cells. When CAR-T cells bind to tumor surface antigen, they proliferate and kill tumor cells. Let us now discuss about the intracellular signaling domains and generations.

The activation of T cells mediated by first generation CARs is accomplished through the tyrosine activation motif on the CD3-zeta chain of the FcR-gamma. The CD3-zeta chain can provide signals for T cell activation and target cell lysis, regulation of IL-2 secretion and, anti-tumor activity in vivo. However, the anti-tumor activity of first-generation CAR modified T cells is limited in vivo and this decreased T cell proliferation ultimately leads to the T cell apoptosis. Second generation CARs incorporate an additional costimulatory signal which amplifies the original “signal 1” derived from the TCR/CD3 complex and increases T cell proliferation and cytokine secretion promoting the secretion of anti-apoptotic proteins.

A commonly used co-stimulatory molecule is CD28 or CD137. To further improve the design of CARs, third generation CARs were developed which include not only the CD3-Zeta and one co-stimulatory domain, but also an additional co-stimulatory signal. Based on these second or third generation CARs, fourth generation CAR T cells co-express some key cytokines or costimulatory ligands such as IL-12, IL-15 and IL-7 or suicide genes which significantly enhance the expansion activity of T cells. The fifth generation CAR T cells has been proposed to knock out the human leukocyte antigen and TCA genes of T cells obtained from healthy donors by gene editing to avoid host immune rejection of graft versus host disease against transplanted CAR T cells as it does not need to be modified according to the patient, this strategy can be used for the treatment of multiple patients.

Summary of CAR T cell generations, so you can see here the first generation, the second generation, third, fourth and fifth generation and you can see the various domains being added up with each generation and at last also you can see the fifth generation which is being done with the help of genome editing. So, in brief, the first generation of CARs contain only a CD3 zeta as well as a well documented intracellular signaling domain. Second, third generation of CARs involve one or two co-stimulatory molecules in addition to CD3 zeta respectively. As well, the fourth generation of CAR-T cells strongly motivates the downstream transcription factor to promote factor to prompt cytokine generation following inter relation between CAR and target antigen. The genome editing technologies such as CRISPR Cas9 have been widely used to construct TRAC (TCR)-deficient CAR-T cells establishing fifth generation of CAR-T cells.

CAR-T cell manufacturing by genome editing.

Many current CAR T cell manufacturing protocols involves ex vivo autologous T cell expansions followed by transduction with a viral vector containing the chimeric receptor sequence. Lentiviral transduction and integration is stable and considered generally safe for clinical trials and FDA approved treatments. However, there are possible risk for malignant transformation of engineered CAR-T cells via insertional mutagenesis for tumor suppressor genes or oncogenes. Lentiviruses integrate semi-randomly in the genome. The CAR transgene can insert in sites with high or low relatively transcriptional activity, leading to variable cell surface CAR expression, generating a suboptimal therapeutic product.

Placing the CAR transgene under control of a strong exogenous promoter may also lead to high constitutive receptor expression. Elevated surface expression and interaction with other CAR receptors can generate ligand-independent tonic signaling in the absence of exogenous signals. Tonic signaling induces both systematic production of cytokines as well as cell profile that drives rapid transition to poor effector functions and T-cell exhaustion. Genome editing tools can replace viral-lentiviral transduction to eliminate the problems associated with it. CRISPR-Cas9 can be used to deliver CAR encoding DNA cassettes to a specific genomic location, allowing for targeted knock-in of the CAR into desired sites.

For example, an anti-CD19 CAR can be directed to the T cell receptor alpha constant (TRAC) locus, resulting in uniform CAR expression, reduced tonic signaling, decreased exhaustion and increased anti-tumor efficacy. Let us examine the strategy of CAR T cell immunotherapy. So here you can see the patients and from this patient T cells are isolated and these are the endogenous TCRs on these T cells and there is gene transfer, then we have these CAR-T cells where you can see the chimeric antigen receptor and this can be subjected to genome editing after which the cells are expanded or proliferated and then transferred back into the patients or the process of adoptive transfer. So peripheral blood T cells isolated from a patient with hematological malignancy is subjected to genetic modification with a relevant CAR that can target the surface antigens of malignant cells. Subsequently the CAR modified T cells are subjected to ex vivo expansion and then administered via adaptive transfer to the patients as already described.

Tumor cells are recognized and killed by CAR-T cells in an HLA-independent manner. Antitumor immunity can be enhanced and optimized. So here is the tumor cell and this is the CAR-T cell and this cell will be killing this tumor cell in an HLA-independent manner. Antitumor immunity can be enhanced and optimized through genome editing such as TALEN, ZFN or CRISPR Cas9 and other gene transfer or editing technologies. Common surface antigen targets for CAR T-cells therapy.

Some of the most commonly used targets for CAR T therapy are surface antigens that has been used in clinical studies as listed below. We have here the surface antigen corresponding to a particular cancer type. For example, CEA present in colorectal adenocarcinoma, fibroblast activation protein found in malignant pleural mesothelioma. diganglioside GD2 in neuroblastoma, glioblastoma, melanoma, osteosarcoma. HER2 is found in HER2 positive sarcoma, mesothelin in pancreatic cancer, IL-13 receptor alpha glioma, mutant alpha-beta-6 integrin pancreatic tumors, epidermal growth factor receptor, lung cancer and breast cancer. Let us now look into the other targets of CAR-T therapy. TCA engineered T cells always target the p-HLA complex. However, the p-HLA complex can also be recognized by CAR-T cells whose scFv for binding was delivered from a TCR-like antibody.

Pule et al. generated Epstein-Barr virus-specific T cells to recognize GD2 and infused these GD2 CAR-T cells into patients to treat neuroblastoma which exhibited moderate anti-neuroblastoma activity. HER2 is thought to be an ideal target for cancer therapy and many strategies have targeted HER2 to successfully treat breast cancer, gastric cancer and other tumors. Morgan et al reported that the infusion of HER2 CAR-T cells to treat metastatic colon cancer caused severe adverse effects, likely due to the large number of third-generation CAR-T cells used and "on-target off tumor" toxicity. The meticulous redesign of the clinical strategies used, including the splitting of the HER2 CAR-T cells infusion, the use of a second-generation CAR construct with severe scFvs, and a reduction in the total number of CAR-T cells effectively improve safety while maintaining anti-tumor efficacy. CAR-T cell treatment has supported appreciated attainment to treat hematological malignancies, including lymphoma.

Chronic lymphocytic leukemia and acute lymphoblastic leukemia. CARs deliver a wider area of functional impacts than transduced TCRs. However, CARs and TCRs have their own set of advantages and disadvantages. Although the flexibility and dynamic range of CARs are striking, existing CARs are restricted to identify cell surface antigens, while TCRs identify both cell surface and intracellular proteins. Nonetheless, antigen processing and presentation of HLA are not required for CARs, making them more applicable than TCRs to HLA diverse patient populations.

There are two primary distinctions between TCRs and CARs that lead to major differences in their function. Firstly, TCRs target peptide molecules. that are bound to MHC molecules expressed on the surface of cells, while CARs target cell surface molecules independent of MHC binding. Secondly, CARs possess all of the molecules required for antigen binding and T cell activation, whereas TCRs are only able to bind to MHC molecules to relay the first signal of T cell activation, meaning that secondary and tertiary signaling is required for T cell activation after the TCR initially binds to antigen. What are the challenges in ACT? The potency of CAR and TCR therapies using autologous engineered T-cells have been well demonstrated by the clinical outcome from the New York esophageal squamous cell carcinoma TCR and CD19 CAR T-cell.

Despite impressive clinical results, plenty of patients are unable to benefit from T-cell therapy due to several reasons. First, the personalized approach of manifesting T-cells is time-consuming and costly, which impedes many patients, especially with rapidly progressive diseases, to make the most of this immunotherapy. Second, during the production process, it is hard to generate enough high-quality T-cells from lymphopenic patients in poor condition. Even if patients get enough immune cells, these cells may fail to complete the whole manufacturing process. Moreover, the risk of manufacturing T cells is always there.

A patient with B-cell leukemia was reported relapsing nine months after receiving anti-CD19 CAR-T cell infusion due to unintentionally transduction of CAR gene into a single leukemic B-cell, as reported by Ruella et al. in 2018. Finally, heterogeneity among autologous CAR-T products contributes to unpredictable and variable clinical activity. Let us now discuss about another important topic, which is tumor antigen escape. Normally, T cells expressing two or more independent CAR molecules have more effective anti-tumor functions than those of T cells expressing a single CAR molecule.

However, the difference is that T cells can be equipped with two or more CARs that recognize different TAAs. Antigen escape remains a major mechanism of relapse and is a key barrier for expanding the use of CAR-T cells towards solid cancers with their more diverse surface antigen repertoires. For some malignancies, it can be difficult to determine whether CAR-T cell therapy against a specific combination of TAAs is safe and effective. Potential methods for overcoming this challenge include enabling CAR-T cells to specifically recognize multiple antigens and respond to lower levels of target cell antigens.

Low levels of infiltration into tumor tissue.

Compared to hematological malignancies, solid tumor CAR-T cell therapy is limited by the ability of CAR-T cells to traffic to and infiltrate solid tumors. The immunosuppressive tumor microenvironment and physical tumor barriers such as the tumor stroma limit the penetration and mobility of CAR-T cells. One strategy to ameliorate those limitations is through the utilization of delivery routes other than

systemic delivery as local administration. 1 eliminates the need for CAR-T cells to traffic to disease sites. 2 limits on target of tumor toxicities as the CAR T cells on target activities directed on tumor cells minimizing interaction with normal tissues.

CAR-T cell associated toxicities.

The toxicities underlying CAR-T cell therapy have been most extensively characterized in patients in the first FDA-approved CAR-T cell therapy, CD19-directed CARs.

23-46 percent of patients displayed severe supraphysiologic cytokine production and massive in vivo T-cell expansion. These toxic levels of systemic cytokine release and severe immune cell cross-examination in some patients result in the following toxicities. Cytokine release syndrome that is associated with supraphysiologic cytokine production and massive in vivo T cell expansion. Second, haemophagocytic lymphohistiocytosis and or macrophage activation syndrome that is a severe hyperinflammatory syndrome characterized by CRS and combinations of elevated serum ferritin and haemophagocytosis renal failure, liver enzymes, spleenomegaly, pulmonary edema and or absence of NK cell activity.

Immune effector cell associated neurotoxicity syndrome (ICANS) i.e. elevated cerebrospinal fluid cytotoxin levels and blood brain barrier disruption.

Strategies to overcome challenges.

To overcome the barriers of limiting wide application of CAR-T cell therapy, multiple strategies have been developed. One of the most feasible and durable approaches is to generate allogenic universal CAR-T cells from healthy donors. Compared with autologous CAR-T cells of the self, allogenic CAR-T cells have many potential advantages including immediate availability of cryopreserved CAR-T cells for patients in urgent need, enough quantity for first infusion or redosing and possible standardization of CAR-T cells producing. Whereas considering the presence of endogenous HLA and TCR on donor T lymphocytes, the biggest challenges of Universal product is the potential risk of alloreactivity and graft versus host disease. Genom editing has been widely used to

generate “off-the self” allogenic CAR T cells that removes alloreactivity and to address other limitations associated with immunotherapy strategies like TCR-T cell therapy.

Thank you for your patient hearing. We will be continuing this discussion in part B of this lecture. Thank you.