Genome Editing and Engineering
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Module - 10
Engineered immune cells for Cancer therapy (I)
Lecture - 02
Engineered immune cells for Cancer therapy (I) -Part B

Welcome to my course on Genome Editing and Engineering. We are discussing about engineered immune cells for cancer therapy. In part A, we discussed about the basics of immune therapy and the tumor microenvironment and we also discussed about the two main immunotherapeutic approaches. So, here let us look into the major categories of immunotherapy. One of the important category is the checkpoint block therapy, where we block the negative regulation of immune checkpoint on immune response to anti-tumor. Another approach is oncolytic immunotherapy, where infecting tumor cells and inducing durable immune responses to destroy cancer is adopted. Some people also deploy cytokines. They help modulate or regulate immune system activity to fight against cancer.

Another process is adaptive cell immunotherapy. It generates a powerful immune-mediated anti-tumor effect through adaptive immune cells. And one of the very promising ones are the cancer vaccines, which act by inducing specific immune response to anti-tumors.

Brief overview of immunotherapy methods: Let us discuss these methods in detail.

Immune checkpoint therapy: Immune checkpoints are molecules of co-inhibitory signaling pathways that act to maintain immune tolerance, which avoid indiscriminate immune attacks. However, they are often utilized by cancer cells to evade immune surveillance. Immune checkpoints inhibitors are designed to reinstate anti-tumor immune responses by interrupting co-inhibitory signaling pathways and to promote immune mediated elimination of malignant cells. The most widely used targets for ICIs are cytotoxic T lymphocyte associated molecule 4, program cell that receptor and program

cell that ligand 1. CTLA-4 is a co-inhibitory molecule expressed on T cells and functions to negatively regulate T cell activation.

PD-1 was discovered to be expressed on the surface of T cells and was originally thought to be involved in program cell death. However, later it was proven to act as a negative regulator of immune responses. The ligand PD-L1 is expressed in normal tissues and regulates immune tolerance by suppressing TCR mediated lymphocyte proliferation and cytokine secretion when binding with PD-1. Abnormally expressed PD-L1 in tumor cells helps to escape immune surveillance. CTLA-4 and PD-1 exert their biological effect at distinct body sites and times during the T-cell lifespan.

Therefore, they complement each other functionally and ensure that T-cell responses preserve self-tolerance while effectively protecting the body from pathogens and neoplasia. Ipilimumab, a CTLA-4 monoclonal antibody, is the first ICI-approved cancer treatment due to its ability to enhance T-cell activation and induce durable responses. Antibodies targeting PD-1 or PD-L1 have also been approved for the treatment of multiple cancers. Studies on CTLA-4 by James and PD-1 by Honjo led them to win the 2018 Nobel Prize in Physiology or Medicine.

The brief anti-tumor mechanism of CTLA-4 and PD-1 or PD-L1 blocking antibodies: In figure A, you can see the tumor microenvironment, the T cell surface is highly inhibited by inhibitory immunoregulatory receptors such as CTLA-4 and PD-1 or PD-L1, which prevents the immune activation of T cells and the killing of tumors. In figure B, you can see the use of PD-1 or PD-L1 or CTLA-4 blocking antibodies can eliminate the immunosuppressive effect of PD-1, PD-L1 or CTLA-4 thereby activating the immune response of T cells to kill the tumors. Let us now discuss about the cytokine therapies.

Cytokines are released by immune and non-immune cells in response to cellular stresses such as infection, inflammation and tumorigenesis. Secreted cytokines enable the rapid propagation of immune signaling in a complex yet efficient manner to generate potent and coordinated immune responses against the targeted designs. Cytokine interleukin-2 expands T cells in vitro and in vivo and thus exerts immunostimulatory properties. As a typical instance of cytokine therapies, the administration of large doses of IL-2 in clinical

applications could lead to cancer regressions in patients with metastatic cancer. Cytokine interferon alpha, IFN alpha, also serve as a classic therapeutic cytokine in cancer treatment.

Interferons comprise a large family of cytokines, among which IFN alpha, a pleiotropic cytokine of type 1 IFN, is a critical determinant of the efficacy of anti-tumor immunity. IFN alpha plays multifaceted roles in tumor control, including directly eradicating tumor cells. through inducing senescence and apoptosis and boosting effective anti-tumor immune responses through the stimulation of DC maturation and the enhancement of T-cell cytotoxicity. IFN-alpha not only activates immune cells directly, but also effectively activates the systemic immune responses by reversing the immunosuppression of effector mesenchymal stromal cells. Clinical studies have proven the therapeutic role of IFN-alpha at high doses in chronic myeloid leukemia and melanoma.

Despite clinical benefits, poor tolerability and severe toxicity hamper further applications of these cytokines as monotherapies. But cytokines are still being investigated in combination with other immunotherapies such as adaptive cell transfer therapy.

Let us now discuss about oncolytic virus therapies. In oncolytic immunotherapy, the therapy is based on oncolytic viruses which can lead to lysis of the tumor cells and the activation of the innate and adaptive immune response by specifically replicating in cancer cells without damaging normal cells. So, we can see here the oncolytic viruses as represented by these figures.

These fights malignancies without dependence on specific antigen expression, which makes it superior to other immunotherapy process. So, these are the oncolytic viruses. So, they enter the immune cells. Here you can see virus-specific reception mediated cell targeting via cell surface antigens, example HER2, may be facilitated.

Then in the second stage, there is a virus replication and the third still there is a cell lysis. So, in the second stage, there is increase in viral replication in tumor cells undergoing rapid cell division. Example, mutations in tumor deliver and here, we exploit the deficiencies in immune pathways. And the fourth stage, there is enhanced apoptosis and or cytotoxicity of the tumor cells. In 2015, a genetically engineered oncolytic herpes

simplex virus Talimogene-Laherparepvec (T-VEC) was approved for the treatment of advanced melanoma by the United States FDA.

Oncolytic viruses stimulate the immune system to recognize cancer cells and activate anti-tumor immunity mainly by inducing immunogenic cell death on them. However, OVs are identified by the immune system as causative agents. and the immune system generates the antiviral response which could diminish the efficiency of the anti-tumor by clearing the virus prematurely.

Cancer vaccines: Cancer vaccine takes advantage of tumor associated antigens (TAAs) or tumour-specific antigens (TSAs) to stimulate the immune system, especially a robust and long-lasting immune response of CD8+ T cells to inhibit the growth, metastasis and recurrence of tumour cells.

According to the clinical use of cancer vaccines, they are divided into two categories: preventive vaccines and therapeutic cancer vaccines. The preventive cancer vaccine aims to prevent tumor occurrence by inducing immune response while the therapeutic cancer vaccines are designed to eradicate tumor cells by inducing or enhancing the tumor specific immunoreactions. The efficacy of the cancer vaccine is primarily dependent on immunogenicity, host immunosuppression, preferential expression of tumor antigens and the delivery of cancer vaccine. The tumor-specific antigens, also known as neoantigens, are ideal cancer vaccine targets due to the highly immunogenic lower risk of self-tolerance, less common tumor antigen deletions, and lower risk of autoimmune reactions.

So far in phase 3 randomness trials there is no other therapeutic cancer vaccine yet showing noteworthy clinical efficacy except sipuleucel-T as reported by Yang in 2022.

Let us discuss about adaptive cell therapy. Advancements in synthetic biology and novel gene therapy techniques. have given rise to advanced cancer immunotherapy methods called adaptive cell therapy or ACT. ACT therapies utilize either the patient's own or donor's immune cells.

The first is known as autologous transfer, second is known as the allogenic transfer. Particularly T cells which are isolated or genetically engineered ex vivo, expanded and re-infused back into patients to eliminate cancer cells and have shown sustained clinical efficacy. Immune subtypes delivered by ACT can include dendritic cell, natural killer cells and T lymphocyte cell-based immunotherapies, each of which is at various stages of preclinical and clinical development as reported by Kumar in 2021. Adoptive cell therapy is followed through three major methods including one using tumor infiltrating lymphocytes, T cell receptors, modified T cells, engineering chimeric antigen receptor cells or CAR T-cells.

Here in this diagram we can see the various adoptive T cell therapy strategies.

Adoptive T cell therapy for treating cancer patients requires ex vivo expansion of autologous T cells for infusion back into the patients. Adoptive T cell transfer of TIL, left side of this figure you can see occurs by first resecting tumor lesions from a patient and then isolating tumor reactive T cells from the The tumour reactive T cells are then expanded ex vivo and then infused back into the patient. So, it makes a full circle journey as you can see in this diagram. Adoptive cell transfer or genetically engineered T cells on the right side occurs by first isolating the peripheral blood, lymphocytes derived T cells from patient's blood, then genetically modifying them to express a specific TCR or CAR. The TCR or CAR engineered T cells are then expanded ex vivo and infused back into the patient. So, here also you can see the full journey from the patient back to the patient.

Tumor infiltrating lymphocyte therapy: In the late 1980s, National Cancer Institute carried out a pioneer study where tumor infiltrating lymphocytes was used in the treatment of metastatic melanoma. Rosenberg and colleagues isolated TILs from biopsy of cancer patient which were expanded under the action of IL-2 and re-infused back into the patient with large dose of IL-2. The objective response rate was 34%. However, the median duration of response was only 4 months and few patients experienced a complete response.

Let us study incorporated lymphodepletion elimination of bodies T cells before ATC therapy where lymphodepletion preparative regimen consisting of 60 milligrams per kg

of cyclophosphamide for 2 days and 25 mg of fludarabine administered for 5 days prior to ACT increased both the rate and the duration of clinical response. In mouse models and in humans, Lymphodepletion prior to cell transfer showed meaningful improvement in the effectiveness of ACTs through enhanced persistence of transferred cells. Studies on mouse suggest that lymphodepletion enhances the efficacy of TILs through elimination of immunosuppressive cells such as myeloid-derived suppressor cells and FOXP3 plus regulatory T cells.

This is the general scheme for the expansion of naturally occurring TILs for use in ACTs. So, you can see the tumors are surgically excised under anesthesia, cut into small pieces or digested enzymatically to obtain single cell suspensions. Tumor fragments are grown individually in high dose of IL-2. This is known as the pre-repeat expansion phase. Under the influence of IL-2, cytotoxic lymphocytes overgrow and kill tumor cells within 2 to 3 weeks. Cytotoxicity of pure lymphocyte cultures are tested by co-culturing IL-2 primed lymphocytes and tumor cells. Individual cultures with high toxicity against target tumors can be rapidly expanded in the presence of irradiated feeder lymphocytes and autoantibody, targeting the epsilon subunit within the human CD3 and IL-2. Using this approach, Rosenberg and colleagues harvested approximately 1011 lymphocytes in approximately 5 to 6 weeks for infusion into patients. Lymphodepletion preparative regimen administered for 5 days demonstrated remarkable outcome in effectiveness of the ACTs. Patients were infused with cells and interleukin-2 at 720,000 IU per kg to tolerance after lymphodepletion.

What are the limitations of TILs? Using the autologous inactive yet potential T cells was the initial idea. However, getting adequate and appropriate healthy tumor-specific T cells is not always possible. In many cancer types, therapy-affected T cells with anti-tumor activity are not present, thus cannot elicit therapeutic responses of teal-based ATC. Preparation of efficient T cells by these method is time consuming and together with intolerability against evolving changes in tumor makes it its applications limited mostly to melanoma. That is why TIL therapy being an effective in even melanoma however it is very limited because many tumor relations are not easily accessible for TIL harvest. To

overcome these problems, advanced techniques of utilizing engineered lymphocytes in ATC was developed subsequently.

TILs directly recognize antigens presented on the surface of tumor cells in the form major histocompatibility complex peptide complexes. Because tumor associated antigen is also expressed on self-tissue, immune tolerance occurs when using TILs exposed to pMHCs derived from TAAs resulting in unresponsive T cells.

Let us now discuss about the TCR modified T cells. Problems associated with TILs and immunosuppressive environment of tumor microenvironment are attempted to be overcome by development of TCR engineered lymphocytes. Treatment with engineered tumor antigen specific T cells has demonstrated significant clinical successes in patients with metastatic melanoma, colorectal carcinoma, synovial sarcoma and multiple myeloma.

TCRs are able to assess the full proteome of the cell. However, they require antigen processing and presentation of peptide targets via the MHC system for functioning. In TCR T-cells therapy, ex vivo expansion of anti-tumor T lymphocytes carried out after they have been genetically modified by the ex vivo insertion of genes, encoding carefully selected TCRs of known specificity and affinity. The autologous peripheral blood lymphocytes are genetically engineered to express a novel TCR that recognizes specific tumor antigens. T-cell receptor engineered effector cells use a naturally occurring TCR to develop T-cell based adaptive T-cell therapy.

This approach has been selected for its ability to recognize tumor specific epitopes presented by the major histocompatibility complex molecules on the tumor cell surface. The T-cell based adaptive T-cell therapy has a potentially broader applicability. as there are far more tumor-specific peptide sequences within a cell and present in MHC than number of tumor-specific cell surface antigens. These intracellular cancer targets are only accessible by TCR-based approaches and not by other immunotherapy techniques such as CAR-based approaches. TCR T cells have also targeted new antigens generated by somatic mutations in tumor DNA, which are more tumor specific, but also less shared by the cancer patients.

ACT can in principle utilize a variety of effector cells, but it is most commonly based on T cells. or natural killer cells derived from the patient and genetically modified.

TCR T cell structure. This is an antigen presenting cell and here we see a T cell. We see different molecules like CD3 epsilon, CD3 delta, gamma. and so on and we see TCR alpha, TCR beta and HLA class 1 and also the peptide antigen.

The TCR is a molecule on the surface of T cells that specifically recognizes and mediates immune responses and consisting of two highly variable heterogeneous peptide chains linked by disulfide bonds. The disulfide bonds are present between the conserved cysteine residues located within the constant region of each chain. In the majority of mature T cells, the TCA consists of alpha and the beta chains. although there is a smaller population of T cells in which the TCR consists of gamma and delta chains as well. Antigen recognition by the alpha-beta TCR is central to the function of the adaptive immune system.

Alpha beta TCR bind to the peptide major histocompatibility complex on the surface of antigen presenting cells. The interaction between an α, β, TCR and a pMHC is highly specific as T cells can distinguish between rare foreign pMHCs and abundant self-pMHC molecules. Neither TCR-chain has intrinsic signaling capacity and activation requires interaction between the TCR and other accessory signaling molecules. A non-covalent oligomeric complex comprised of TCR and CD3 signaling molecules, as shown here, initiates signaling activities on binding a cognate peptide MHC complex on a target cell and enables antigen-specific tumor cell lysis. Alpha-beta-TCRs activate the TCR signaling pathway by binding to the major histocompatibility complex on tumor cells or antigen presenting cells which then activates a series of intracellular proteins including CD3-Zeta, 70-KD Zeta-associated protein (ZAP70) and nuclear factor of activated T cells 2 thereby mediating T cell immune function.

Selection of an appropriate antigen for the development of safe and effective TCA based adaptive therapy: The selection of the antigen and the cognate TCR are of vital importance. Candidate target antigens that are used for TCR engineered T cell treatment

require three features. If they are to be utilized, they must be selectively expressed in tumors and not in normal tissues.

They are related to oncogenes. They are able to evoke a T cell response. Neo-antigens demonstrate many of the features considered ideal. However, the wide diversity of somatic mutational profiles combined with high HLA-1 polymorphism makes vast majority of tumor neo-antigens personalized and not shared among cancer patients. Tumor-associated antigens are peptides that originate from endogenous wild-type proteins whose expression is elevated in tumors but limited in magnitude or in spatial expression in healthy tissues. TCR T cells are constructed by transferring a TCR gene sequence that specifically recognizes tumor antigens into T cells through genetic engineering, so that the T cells have the ability to specifically kill tumor cells.

TCR T-cells can recognize not only specific antigens on the surface of tumor cells but also intracellular antigens which allows TCR T-cells to recognize a wider spectrum of target antigens. TCR T-cells are predominantly engineered to express one transgenic TCR alpha-chain and one transgenic TCR beta-chain in addition to or in place of their endogenous chains. The cloning of TCRs from tumor infiltrating lymphocytes can redirect bulk T cells to have a tumor specificity. On binding of transgenic TCR to a therapeutically relevant peptide MHC of interest for example, the 9-mer peptide derived from amino acids 157 to 165 of the highly immunogenic cancer testis antigen NY-ESO-1, which is presented by HLA-A, natural TCR signaling activates T cell function and expansion. For the development of safe and effective TCR based adaptive therapy, the selection of the antigen and the cognate TCR are of vital importance.

Target antigen should be selectively expressed in tumors and not (or only at very low levels) expressed in normal tissues. Consequently, a specific and selective TCR with sufficient target affinity and minimal cross-reactivity against other peptides is needed. In addition, an effective and robust T cell transduction and expansion process must be developed that allows the reliable delivery of a potent and safe immunotherapy product to the patient. The transduction efficiency is of paramount importance as there is a significant patient to patient variation in the number of T cells collected for manufacture of the ACT product.

Identification of TAA neo antigens: Both tumor cells and tumor infiltrating lymphocytes are isolated from the patient. The isolated tumor cells are subsequently subjected to gene sequencing: example whole genome sequencing, mass spectrophotometer analysis, and/or bioinformatic analysis promoting the identification of tumor-specific TAA neoantigens. To validate the immunogenicity of the identified TAA neoantigens APCs expressing the identified TAA neoantigens are co-cultured with the TILs isolated from this patient. The specific population of TILs bearing TAA neoantigen-specific tissues which exhibit cell proliferation or cytokine secretion in response to the stimulation of APCs expressing tumor-specific neoantigens can be isolated and the neoantigen-specific TCS can then be cloned successfully. Subsequently, the cloned TAA neoantigen-specific TCS are transduced into the patient-derived T cells, generating genetically modified neoantigen-specific T cells via ex vivo activation and expansion.

The modified T cells bearing the neoantigen-specific TCS can be adoptively transferred to the patient and target tumor cells bearing tumor-specific TA and neoantigens with high specificity for elimination. In addition, the intratumor heterogeneity can be dissected by the single-cell sequencing or other technologies which can facilitate the identification of clonal neoantigens and thus improve T cell immuno-reactivity.

Editing methods for genetically engineered T lymphocytes: Tumor antigen-specific TCR repatories identified by next-generation sequencing can be used to genetically engineer T lymphocytes for the TCR-T therapy. Most TCR-based gene therapy approaches rely on the ex vivo transduction of T cells with viral vectors such as the retroviral vectors derived from gamma retroviruses, lentiviral vectors, adeno-associated virus.

Several non-viral gene editing methods have been developed such as mRNA electroporation to minimize the risk of viral element persistence. Recently CRISPR-Cas9 genome targeting system has also been used for rapid and efficient insertion of large DNA sequences at predetermined sites in the genomes of primary human T cells while preserving cell viability and function. Tumor cells presenting new antigen-derived peptides can be recognized and killed by genetically modified T cells, bearing the responsible neoantigen specific TCR as shown in this figure.

So, this is the neoantigen specific TCR in this cell. Genome editing and gene transfer technologies and other alternative measures can be utilized to modify the components of other alternative pathways for immune enhancement, ultimately providing an optimized approach to improve TCR T cell based therapies. So, here there are the receptors in this TCR T cell and genome editing through any of these tools, ZFN, TALEN and CRISPR can be done. And, here the neoantigen specific TCR expression and finally, the neoantigen presentation is shown in this particular figure and this helps in the killing of the tumor cells.

This is a schematic view of TCA based adaptive T cell therapy. There are various stages 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. So, in the first stage you can see the HLA typing, then tumor biopsy, biomarker profiling, leukapheresis. Then, PBMC pre-activation using anti-CD3 and CD28 antibodies, then there is a healthy donor, TCR characterization and modification, lentiviral vector encoding novel target specific TCR and generation of TCR transgenic T cells and this T cell enrichment and expansion and then finally, treatment and monitoring. If HLA is A*02:01 type, a tumor biopsy is performed to screen the tumor tissue for the expression of the targeted antigen followed by leukapheresis. The PBMCs from patient leukapheresis are isolated and pre-activated using anti-CD3 and CD28 antibodies as already told. A target specific TCA is isolated from the healthy donor, characterized and modified and then a lentiviral vector is constructed and used to transfer the target specific TCR in the T cells.

The activated PBMCs are transduced with a lentiviral vector encoding the target specific TCR. The transduced T cells are expanded to large numbers in 3 to 5 days and are frozen. Upon completion of the release testing, the T cells are ready to be infused. The patients are typically treated with lymphodepletion followed by T-cell product infusion followed by low-dose interleukin-2. Patients are monitored for as long as 15 years to observe for delayed adverse events following exposure to the investigational gene therapy products.

Here are some of the references which were utilized for preparation of this lecture. So, you can go through the relevant literature for getting more details about some of the concepts that has been presented in this lecture. Thank you.