


Cancer Immunotherapy and CAR T Cells

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ABSTRACT

Within the past decade, radical changes of idea emerged from the works of research scientists, medical experts, and oncologists. Instead of treating cancers with man-made chemicals or chemotherapy and radiotherapy, or surgically remove the tumor, we should unleash the power of our own body immune system. Indeed, such approach has borne fruits and benefited at least for some cancer patients. This review describes various types of immunotherapy known to date, with its beneficial effects, albeit some with serious harmful effects. To understand how immunotherapies work, we also review some biologic characteristics of cancer cells that let them evade attacks from our immune system; cancer microenvironment that works in favor or protecting the growth of cancer against our immune system will also be discussed.

Keywords: Cancer, immune system, immunotherapy

ABSTRAK

Hanya dalam jangka 10 tahun terakhir, telah terjadi perubahan radikal hasil penelitian para ahli, ahli medis, dan ahli onkologi. Di samping cara klasik penyembuhan kanker seperti obat-obat kimia sintetik atau kemoterapi dan radioterapi atau pembedahan, kini para ahli telah menggunakan sistem imunitas tubuh penderita sendiri. Cara ini telah terbukti dalam pengobatan beberapa pasien kanker. Dalam ulasan ini kami akan memaparkan beberapa immunoterapi saat ini dengan hasil baik meskipun masih terdapat beberapa efek samping berbahaya. Untuk memahami bagaimana imunoterapi bekerja, akan diulas dan dijelaskan sifat-sifat biologi sel kanker, dan bagaimana kanker sel bisa menghindari serangan sistem imun; dan lingkungan mikrokanker *(cancer microenviroment)* yang melindungi kanker dari sistem imun. **Khing S. Ong, Zack ST. Lim, Boenjamin Setiawan. Imunoterapi Kanker dan Peranan *CAR T Cells.***

Kata kunci: Immunoterapi, kanker, sistem imun

INTRODUCTION

Science magazine named "cancer immunotherapy" as 2013's breakthrough of the year[1] indicating not only the importance of cancer immunotherapy (CI), but also the significant successes that have been accomplished over the years. Some of the earlier discoveries validate the potential of CI, including the seminal work by James Allison of University of Texas, MD Anderson Cancer Center in Houston, showed that antibodies against CTLA-4 (cytotoxic T-lymphocyte antigen 4) can wipe out tumors in mice model,[2] and the development of promising cell therapy using engineered patient's own T cells, the so-called CAR T lymphocytes by Carl June of University of Pennsylvania Abramson Cancer Center[3] and Michel Sadelain of Memorial Sloan Kettering Cancer Center in New York City[4] independently. Based on these two cornerstone findings, research on CI has flourished and grown tremendously in the past decade.

HISTORY OF IMMUNOTHERAPY

It is common knowledge that throughout history many cases of tumor growth regression were reported after an infectious or high febrile episode. In late 18th century, two German physicians[5,6] independently reported cases of tumor regression in patients after erysipelas infection (a *Streptococcus pyogenes* infection). More serious consideration of infection and its consequences of immune activation that could cause tumor regression was reported by William Coley of Memorial Sloan Kettering in 1908.[7] Coley observed long term regression of sarcoma after erysipelas infection, and started a project with injection of his "Coley Toxins" (heat inactivated bacteria) into patients with inoperable cancers, resulted in significant regression or even cured in over 1000 patients, most of them were with sarcoma.[7] This success has generated excitement, but it was short lived because of the lack of further systematic research. In 1959, Old, *et al*, reported antitumor effects of BCG (Bacillus Calmette-Guerin) in mouse model.[8] In 1976 Morales, *et al*, showed the effectiveness of BCG in the treatment of superficial bladder cancer.[9]

Increased incidence of cancers or tumors in immune deficient patients suggests the important role of immune function in the development of cancer. High incidence of various types of cancers have been shown in AIDS (acquired immunodeficiency syndrome) patients.[10]

CANCER BIOLOGY AND ONCOIMMUNOLOGY

Hanahan and Weinberg in their paper: "Hallmarks of Cancer"[11] assimilated a growing volume of research results and theoretical insight into eight characteristics that cancer

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cells must acquire in its development into a cancer or tumor. They are:

1. Acquiring the ability to stimulate its own growth by sustaining proliferative signaling.
2. Ignoring signals from oncogenes and warning tumor suppressors to slow down.
3. Resisting cell death
4. Inducing angiogenesis.
5. Activating invasion and metastasis.
6. Enabling replicative immortality (work against telomere's limitation).
7. Reprogramming energy metabolism
8. Evading immune destruction.

Underlying cause of these hallmarks is the genomic instability of cancer cells. Each cell in our body experiences more than 20,000 DNA damages each day,[12] which are normally repaired by DNA repair pathways without any serious consequences.[13] Cells which are not repaired can acquire malignant changes, however these are normally killed by immune cells through the immune surveillance system.[14] Some malignant cells are able to evade this immune surveillance by altering their characteristics as well as the cells in their microenvironment.

Immune surveillance is now a part of the broader concept of immunoediting which consists of three phases: elimination, equilibrium and escape phase. In the elimination phase, tumor antigens are picked up by dendritic cells which then in turn present it to the T lymphocytes and created a tumor specific CD4+ and CD8+ T lymphocytes that kill the tumor cells. If the elimination is incomplete, and the remaining tumor cells are unable to progress then the equilibrium phase sets in. In the escape phase, immune system loses the control and tumor cells will grow and metastasize by expressing fewer antigens on their surfaces or losing their MHC class I expression.[15] They may also protect themselves from T cell attack by expressing immune check point molecules on their surfaces like normal cells. These immune check point molecules are part of the normal negative feed-back loop that control excessive tissue damage from inflammation by down regulating or suppressing T cells.[16]

A malignant cell can have more than 11,000 genomic mutations leading to expression of many new tumor associated antigens (TAAs) which include products of mutated oncogenes, tumor suppressor genes, aberrantly expressed protein, antigens produced by oncoviruses, oncofetal antigens, and cell type-specific differentiation antigens. These new TAAs are presented on the MHC molecules on the cell surface. Recognition of this antigen-MHC complex alone is insufficient to elicit an activation of a T cell response, it requires an additional co-stimulation signals provided by the engagement of CD28+ receptor on T cell with B7 ligand molecules (two of which are CD80 and CD86) on the antigen presenting cells. This CD28 receptor and B7 ligand combination or as it is also called "immunological synapse" stimulate proliferation and function of the T cells. Some of these "synapses" are inhibitory, such as in PD-1/PD-L1 and CTLA-4/B7 check point inhibition that will be discussed in different section.

Cancer Heterogeneity

Cancer is a dynamic disease. During the course of disease, cancers generally become more heterogenous and include a diverse collection of cells with distinct molecular signatures and different level of sensitivity to treatment. This heterogeneity might result in a non-uniform distribution of genetically distinct cancer cell subpopulation across and within disease sites (spatial heterogeneity) or temporal variation in molecular make up of cancer cells (temporal heterogeneity).[17] In T cell acute lymphoblastic leukemia in mouse model, researchers found that cancer cells, even genetically identical ones, may behave differently depending on their location in the body, because cells' ability to grow and survive is influenced by the tissue microenvironment. The implication of all is that therapy might be effective against some tumors in some body sites but not in others.[18]

Tumor Microenvironment

The concept of tumor microenvironment is developed based on the view that cancers are not just a mass of malignant cells, but a complex organ to which many cells are recruited and corrupted by cancer cells. Interaction between malignant and non-malignant cells often has tumor promoting effects at all stages of carcinogenesis.[19] Beside malignant cells, tumor microenvironment contains immune system cells such as T and B lymphocytes, natural killer cells, tumor associated macrophages, myeloid derived suppressor cells, dendritic cells, and tumor associated neutrophils. In addition, there are cancer associated fibroblasts, adipocytes, vascular endothelial cells. All these non-malignant cells are within an extra cellular matrix scaffold and consist of more than 50% of the mass. Various chemokines and cytokines are released as a results of intercellular communications, one of them is cytokine receptor interleukin 6 (IL-6), a known tumor promoter. Antibody against chemokine receptor CCR-4 seems to switch macrophages from tumor promoting to being tumor inhibiting with anticancer effects.[20] For metastasis to occur, the tumor has to activate its microenvironment particularly the nearby blood vessels. This process appears to involve chemokines including CCL2 and CCL5.[21] Chemokines not only recruit leukocytes or inflammatory monocytes, they also act as signaling molecules for activation of endothelial cells that are important in metastasis.

CANCER IMMUNOTHERAPY

As discussed in the prior section, cancer cells have various ways to evade detection and destruction by the host immune system. To overcome this, CI uses various approaches to strengthen the immune system, and or help it defeat cancer's defenses against attack from immune activation.

I. DENDRITIC CELLS AND CANCER VACCINES

Cancer vaccines are unlike regular vaccine, such as measles or small pox vaccine that induces host's immunity against the intended disease for long period or even for life, instead cancer vaccine is actually a "tool" to stimulate and activate host's immune system to fight against cancer. Dendritic cell first described by Ralph M. Steinman is an antigen presenting cell[22] which educates other immune cells what to attack and makes up as core of therapeutic vaccines against cancer. Steinman himself was diagnosed with pancreatic cancer, he and a team of his colleagues turned to a new dendritic vaccine to treat his disease. His colleagues believed that the vaccine helped to prolong his life, however he died just three days before winning the Nobel Prize for his works on dendritic cells. Therapeutic vaccines with dendritic cells are made by first extracting the dendritic cells from patient's blood and exposed them directly to cancer antigen or genetic material extracted from patient's own


tumor (personalized antigen), to kick off a tumor targeted immune response. Stimulatory compounds are often added, in case of FDA approved vaccine Sipuleucel-T (Provenge), the dendritic cells are cultured in the lab with prostatic acid phosphatase antigen and GM-CSF (granulocytes-macrophages colony stimulating factor) as stimulating compound, to increase the number and maturation of these dendritic cells before they are re-injected back into the patient.[23]

Other methods have been developed by various institutions and pharmaceutical companies for similarly antigen specific cancer vaccines:

1. Oncophage Vaccines from Antigenics (New York). Oncophage is based on HSPs (heat shock proteins). HSPs act as chaperones for proteins and peptides, and are found externally only in case of cellular necrosis which is present in most tumors. The complex of HSP 90DK beta member 1 (HSP90B1) and associated peptides from patient's tumor are taken up by dendritic cells and triggering a up-regulation of B7 expression that sends a strong danger signal to immune system by activating the killer T cells.[24]

2. In 2009, Harvard research group developed an implantable cancer vaccine, using plastic disc 8.5 mm in diameter made of FDA approved biodegradable polymer impregnated with tumor specific antigen. The discs are highly permeable to immune cells and their released cytokines which recruit dendritic cells where they are exposed to specific antigen of the targeted tumor. The dendritic cells then direct the T cell to hunt and kill the tumor cells.[25]

3. Washington University researchers used tumor missense mutation (missense mutation is when the change of a single base pair causes the substitution of a different amino acid in the resulting protein. This amino acid substitution may have no effect, or it may render the protein nonfunctional) as a source of patient's specific neoantigens, this tumor encoded amino acid substitution (missense mutation) occured in some melanoma patients. Researchers showed that dendritic cells vaccine increased naturally occurring and revealed new HLA class I restricted neoantigens in these patients with advanced melanoma, and the neoantigens created by missense mutation can serve to generate a high frequency responses after cancer patients received the therapeutic vaccine.[26]

4. Researchers from EPFL (Ecole Polytechnique Federale de Lausanne) have created artificial receptors called EVIR (extracellular vesicle-internalizing receptors) which enable dendritic cells in the vaccine to selectively and efficiently capture antigen from patient's tumor. EVIR were inserted into dendritic cell where it recognizes protein on exosomes. Exosomes are profusely released by tumor and containing a variety of tumor antigens. By capturing exosomes from tumors, EVIR helps the dendritic cells to precisely acquire tumor antigens from cancer cells and present these antigens more efficiently to T killer cells.[27]

5. Researchers at Stanford University School of Medicine used a non-customized approach called *in situ* vaccination, where immunoenhancing agents were injected locally into one site of tumor and triggering a T cell immune response locally which could then also attack cancer throughout the body. The enhancing agent used was a combination of CG-enrich oligonucleotide (CpG) – a Toll-like receptor 9 (TLR9) ligand and anti-OX40 antibody. Low dose of CpG injected into tumor induce the expression of OX40 on CD4+ T cells in the microenvironment of tumors. An agonistic anti-OX40 antibody can then trigger a T cell immune response.[28]

II. IMMUNE MODULATING AGENTS

These agents include proteins, bacteria and drugs that normally help regulate or modulate activities of the immune system:

1. Cytokines
   Two types of cytokines are used to treat patients with cancer: Interferons (INFs) and interleukins (ILs). A third type called hematopoietic growth factors is used to counteract some of the effects of certain chemotherapy regimens. INF-alpha can enhance patient's immune response to cancer by activating natural killer cells and dendritic cells.[29] IL-2 - also called T cell growth factor - is produced by activated T cells, it increases proliferation of killer T cells and natural killer cells, and promotes production of antibody by B cells leading to enhanced and targeted anti-cancer immune response. Hematopoietic growth factors are naturally occurring cytokines that promote the growth of various blood cells population that are depleted by chemotherapy. Erythropoietin stimulates formation of red blood cells, IL-11 increases platelet production and G-CSF and GM-CSF both increase the numbers of white blood cells including the cancer fighting T cells.

2. Bacillus Calmette Guerin (BCG)
   This weakened form of live tuberculosis bacterium has been known as potent adjuvant that can stimulate a general immune response. As mentioned earlier, BCG has been successfully used to treat superficial bladder cancer,[9] the exact mechanism of its anti-cancer effect however is not fully understood.

3. Immune-modulatory Drugs (also called Biological response modifiers).
   These are strong modulators of immune system, including thalidomide, its derivatives lenalidomide and pomalidomide, and imiquimod.[30] Thalidomide and its derivatives stimulate the immune system by promoting the IL-2 secretion and inhibit tumor angiogenesis. Imiquimod, a small molecule drug of immune system modifier, act as TLR-7 (toll- like receptor[30] agonist. It is mainly used as cream for skin warts or superficial basal cell carcinoma. It causes cells to release INF-alpha, IL-6 and TNF-alpha (tumor necrosis factor-alpha).

III. THERAPEUTIC ANTIBODIES

These antibodies are made in the lab that are designed specifically to interact with and block a specific molecular target that is necessary for cancer cell growth. They may interfere with a key signaling that promotes the growth of cancer and or alert the immune system to destroy the cancer cell it attached to. Examples are rituximab and ofatumumab both target CD20 on the surface of B lymphocytes, and alemtuzumab targets CD52 on lymphocytes.[31] Majority of these antibodies

are monoclonal antibodies (name ends with –mab). These antibodies can also be linked to a toxic substance such as bacteria toxin, small molecule drug, light sensitive chemicals (photoimmunotherapy), or radioactive compound (radioimmunotherapy) and called "antibody-drug conjugate". Example, ado-trastuzumab emtansine (Kadcyla) that kills cancer cells that express HER2 marker on their surface.

Bispecific Antibody: These antibodies are designed with selective targets proteins found on several types of cancer cells such as ROR1 and at the same time they also bind to T cells and activate the T cells to kill the cancer cells.[32]

IV. IMMUNE CHECKPOINT INHIBITORS

Immune response is regulated by a balance between stimulatory and inhibitory signals. These signals are collectively referred to as "immune checkpoints" which are necessary for maintaining self-tolerance and protecting host from tissue damage. Activated T cells are primary mediators of immune effector functions, they express multiple co-inhibitory receptors such as LAG-3 (lymphocytes activation gene 3), PD-1 (programmed cell death protein 1) and CTLA-4 (cytotoxic T lymphocyte-associated protein 4). These immune checkpoint molecules modulate T cell responses to self proteins as well as to chronic infections and cancer antigens, pathways utilized by these checkpoint protein are unique and non-redundant.[33]

CTLA-4 and PD-1:

CTLA-4 is a homologous transmembrane glycoprotein of the immune co-stimulator protein CD28. CTLA-4 plays a key role in developing peripheral tolerance to self proteins by neutralizing the function of CD28.[34] Following engagement of the T cell receptor (TCR) with cognate antigen, CD28 provides the second signal for T cell activation by binding to CD80 (B7-1) and CD86 (B7-2) proteins on antigen presenting cells. CTLA-4 bind these B7 proteins with high affinity and therefore out-compete CD28 for the binding.[35] Inhibition of CTLA-4 enhances immune responses that are dependent on CD4+ T helper cells. In addition, CTLA-4 blockade lead to inhibition of the immunosuppressive function of Tregs or regulatory T cells.[36]

Stimulatory checkpoint molecules are members of TNF (tumor necrosis factor) receptor superfamily and includes CD27, CD40, OX40, GITR and CD137. Another two belong to B7-CD28 superfamily – CD28 itself and ICOS (inducible T cell co-stimulator). Inhibitory checkpoint molecules include Adenosine A2A receptor, B7- H3 (CD276), B7-H4 (VTCN1) expressed in tumor cells and tumor-associated macrophage and play a role in tumor escape, CTLA-4, IDO (indoleamine 2,3 deoxygenase) that are known to suppress T and NK cells and activate T-regs and myeloid derived suppressor cells and promote tumor angiogenesis,[37] KIR (killer-cell immunoglobin-like receptor) that is a receptor for MHC Class I molecule on natural killer cell, LAG3, PD-1, TIM 3 (T cell immunoglobin domain and Muccin domain 3) expressed on CD4+ T cells.

The two pairs of inhibitory receptor/ligand which received the most attention are CTLA-4/B7 and PD1/PD1-L1. CTLA-4 has high binding affinity to B7 causing inhibition of proliferation and secretion of IL-2 by T cells. PD-L1 and PD-L2 are members of B7 family. Unlike CTLA-4, PD-L1 does not interfere with co-stimulation but generates signals that prevent phosphorylation of key signaling intermediates in T- cell which reduces their activation. While B7 is expressed in dendritic cells, macrophages and B cells, PD-L1 can be expressed on many cell types, including T- cells, epithelial and endothelial cells, and tumor cells after exposure to interferon-gamma. PD-L2 is primarily expressed on dendritic cells and monocytes but can be induced in a wide variety of other immune cells and non-immune cells.[38]

CTLA-4/B7 synapse acts earlier than PD-1/PD-L1 in the immune response because it stops potential autoreactive T cells at the initial activation of the naïve T cell.

PD-1/PD-L1 pathway functions at later effector phase in the periphery and protects the cells found there including tumor cells which express PD-L1 from attack by T cells.[36] PD-1/PD-L1 pathway represents an adaptive immune resistance mechanism that is exerted by tumor cells in response to endogenous anti-tumor activity.[39]

LAG-3: LAG-3 also known as CD223 is a homologous of CD4. Similar to CD4 the only known ligand for LAG-3 is the MHC class ii molecule. Like CTLA-4 and PD-1, LAG-3 is also critical for dampening overt T cell immune responses. LAG-3 specifically inhibits CD8+ effector T cell functions and can enhances the suppressive activity of Tregs.[40]

Antibodies to Immune Checkpoint Molecules:

As mentioned earlier,[2] CTLA-4 blocking monoclonal antibodies (mabs) could treat tumors in mice model. These mabs are known as "immune checkpoint inhibitors" (ICIs) which actually are anti-ICIs, and involve antibodies generated against CTLA-4, PD-1 or its ligand PD-L1 to reverse the exhaustion of cytotoxic T cells.

Inhibitor Antibodies against CTLA-4:

Ipilimumab is the first anti-CTLA-4 drug developed. It is a humanized IgG1 kappa mab that antagonizes CTLA-4 and inhibit its ligand binding.[41] Two phase III trials in patients with advanced melanoma showed that ipilimumab improved overall survival by several months and led to its approval by FDA and European Medicine Agency.[42] Ipilimumab is still being evaluated for other indication of application and combination therapy with other ICIs antibodies. A second anti-CTLA-4 antibody being evaluated is Tremelimumab, a fully human IgG2 mab. A phase III trial comparing tremelimumab to chemotherapy (dacarbazine/temozolomide) in patients with advanced melanoma, tremelimumab did not showed significant survival advantage over chemotherapy.[43] However, trial of combination therapy of tremelimumab and durvalumab an anti-PD-L1 antibody in locally advanced or metastatic non-small cell lung cancer (NSCLC) patients demonstrated a significant anti-tumor activity independent of PD-L1 status.[44]

Inhibitor Antibodies against PD-1/PD-L1:

Several anti-PD-1 and anti-PD-L1 have been developed. Nivolumab, pembrolizumab and pidilizumab are against PD-1. Atezolizumab (MPDL3280A), durvalumab MEDI4736), BMS-936559, and MSB0010718C are against PD-L1. A PD-L2 fusion protein that depletes PD-1 positive T cells is also under development.[45] Clinical studies targeting PD-1/PD-L1 have shown higher response rates than in CTLA-4 blockade studies. Further more, PD-1/PD-L1 blockade in general results in less severe side effects. Fatal pneumonitis was observed


in 1% in an anti-PD-1 antibody clinical trial.[46] A phase III trial comparing nivolumab (anti-PD1) to standard chemotherapy in melanoma patients who did not response to ipilimumab demonstrated a 32% overall response rate with nivolumab compared to 11% with chemotherapy.[47] Pembrolizumab another anti-PD-1 also showed significant efficacy in trial and is approved by FDA for melanoma. Targeting PD-L1 has also showed impressive clinical results. Atezolizumab showed consistent results in patients with NSCLC compared to chemotherapy and is FDA approved for metastatic NSCLC.[48]

Durvalumab (anti-PD-L1) is currently in a clinical trial as monotherapy for metastatic urothelial cancer and showed manageable safety profile and significant clinical activity.[49]

Overall, above treatments with various mabs against immune checkpoint inhibitor suggest encouraging results for future studies, particularly with combination strategy.

V. ADOPTIVE CELL TRANSFER (ADOPTIVE CELL THERAPY).

Adoptive cell transfer (ACT) is a rapidly emerging CI by using patients own immune cells to treat their cancer. There are several types of ACT: tumor infiltrating lymphocytes (TILs), T cell receptors (TCRs) and chimeric antigen receptor T cells (CAR-T Cells).

Tumor Infiltrating Lymphocytes

T cells isolated from tumors and expanded ex-vivo have been used for melanoma over the last two decades.[50] TILs can target tumor neo-antigens, providing a rational to target tumors with high mutational load such as melanoma and lung cancer.[51] However, TILs have limited usage because TILs manufacturing is complex and it depends on the endogenous T cell receptor (TCR) for cancer recognition.

T Cell Receptor

The TCR is unusual in that the antigen receptor recognized peptide and self MHC molecules. An advantage of TCR is that a vast array of distinct peptides can be recognized including specific tumor mutations. There are two limitations of TCR as a recognition modality of T cells, because it required MHC matching to each patient, and the affinity of TCR for cancer target is low (in micromolar instead of nanomolar).

CAR-T Cells Therapy

Until recently, CAR-T cells therapy has been limited to small clinical trials, largely in patients with blood cancers. In 2017 two CAR-T cell therapies were approved by FDA, Tisagenlecleucel (Kymriah) for children with acute lymphoblastic leukemia (ALL), the other Axicabtagene ciloleucel (Yescarta) for adults with advance large B cell lymphomas. Chimeric antigen receptors or CARs consist of an extracellular antigen-recognition domain, which is usually an antibody single-chain variable fragment (scFv), but can also be a peptide or protein linked to an intracellular signaling domain usually the CD3zeta chain of T cell receptor. The extracellular portion of CAR allowed the recognition of a specific antigen by T cell and subsequently the signaling domains stimulate T cell proliferation, cytolysis and cytokine secretion to eliminate the target cell.

Patient's own T cells (or sometime from allogeneic donor) are isolated, activated and genetically modified to generate CAR T cells, which are then infused back into the same patient. This approach carries a very low risk of graft versus host reaction and enables lipid, protein and carbohydrate antigens to be targeted by T cells in an MHC-unrestricted fashion.[52] One limitation of CAR T cell therapy is that it required extracellular surface target on the tumor cells, this is why CAR-T cell therapy targeting CD19+ cells is very successful in treating B cell malignancies because of its high level of CD19 on their surface. As normal B cells also express CD19 on their surface they could also be wiped out and causing a B cell aplasia, however this aplasia can be overcome by antibody replacement treatment (IVIG).[53] These results demonstrate that CAR-T cells can have on target off-tumor effects, a feature that may be mitigated by the development of on-off switch CAR-T cell, with more sophisticate design of recognition receptor that make T cells effectively recognize target tumor cells yet discriminate against normal cells.[54]

First generation CARs utilize CD3zetta signaling chain which provide an activation Signal termed signal 1. First generation CAR T cells showed limited efficacy in clinical trials, probably owing to activation-induced cell death of transplanted T cells or a lack of long term T cells expansion.[55]

Second generation CARs use first generation CARs as backbone and included an additional co-stimulatory signaling domain termed signal 2 to provide an additional signal to optimally activate the T cells. Second generation CAR T cells specific for CD19 that include both CD3zeta and CD28 signaling have been demonstrated to have enhanced persistence and proliferation compared to first generation CD19 specificm CAR T cells.[56]

Third generation CARs contain a CD3zeta domain and two co-stimulatory domains: CD28 or 4-IBB (CD137) and OX40 (CD134). In preclinical studies, third generation CAR T cells have demonstrated superior anti-tumor efficacy compared with second generation CAR T cells.[57] Clinical trial is currently being launched at Baylor College of Medicine, Texas. In solid tumor, the effects of CAR-T cell are much weaker than in blood cancers, to push CAR-T cells forward rational combination with other CI seems warranted.

Side effects of CAR-T cell therapy:
The most frequent adverse effect is cytokine release syndrome (CRS), a rapid and massive release of cytokines into bloodstream which can lead to high fever and precipitous drops of blood pressure. CRS however can be managed with standard supportive therapy and tocilimumab a mab that blocks high level of interleukin-6 secreted by T cells and macrophages in response to inflammation.

Another side effect: neurotoxicities causing confusion or seizure like activity has also been seen in most CAR T cell therapy trials. In mouse model, a recent study of humanized mice with leukemia burden, CAR-T cell mediate clearance of cancer and triggered high fever and elevated IL-6 level (hallmarks of CRS), the syndrome was prevented by monocyte depletion and blocking IL-6 receptor with tocilizumab. However tocilizumab fail to protect mice from delayed neurotoxicity characterized by meningeal inflammation. Instead, IL-1 receptor antagonist – Anakinra abolished both CRS and neurotoxicity.[58]

SUMMARY

1. The explosive development in the past decade on CI suggest a possible beginning of the end of cancers.

2. Among the armament against cancers,

dendritic cell-cancer vaccine seems to have the most potential and may be more cost effective for patients.

3. Rational combination therapy strategy need to be expanded not only among CI modalities but also combination of CI with standard chemotherapy or radiotherapy to reduce side effects of those conventional therapies.

4. Genetic instability is one of the foundation that causes high frequency mutations in cancer cells. With the recent advances in gene editing technology like CRIPR Cas9 and the next generation sequencing technique that makes the genome wide association study easier, may be it is noteworthy for researches to focus also on developing ways to stabilized genes of cancer cell, in hoping that could make them a "fix" target (not evading target by mutation) for immune cells to attack.

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