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Immunotherapy of Main Subtypes of Leukemias

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Abstract

Cancer afflicts millions of people globally each year, and leukemia is a group of blood cancer that consists of four main subtypes, including acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). Although the specific mechanisms of these subtypes of leukemia are not identical, the cardinal principles are alike, where abnormal blood cells accumulate in the bone marrow and affect other cells' normal function. Leukemia owns unique methods to circumvent the immune system's defense and thus over numbers the regular cells in the bone marrow at last. Nevertheless, effective immunotherapies can be developed by focusing on the mechanisms of different types of leukemia. This study introduced several immunotherapies against leukemia, including Anti-CD47 Immune Checkpoint Blockade, STING Agonists, Anti-CSF1R Blockade, Chimeric Antigen Receptor T-Cell Therapy, DC-CIK Immune, and NK Cell Immunotherapy. Each therapy aims at unique factors in order to inhibit the burgeoning of leukemia cells; therefore, treatments can be exploited by humans to enhance the effect of these immunotherapies to achieve the objective of curing leukemia. We also believed that the combination of these imunotherapies might lead to better results in the anti-leukemia process.

Keywords

Cancer, Leukemia, Immunotherapy, Mechanisms, Combination.

1. Introduction

Cancer is a group of diseases that involves abnormal cell growth and may invade other tissues of the body, causing humans' death eventually (Cancer, WHO, 2018) [1]. More than 100 types of cancer have been found, and they caused 8.8 million deaths (15.7% of deaths) annually in the whole world (GBD 2015 Mortality and Causes of Death, Collaborators, Lancet, 2016). Cancer will form subsets of neoplasms where abnormal cells undergo intractable spreading (Weinberg RA, 2020). Potential causes of cancer include genetic mutations, radiation, infection, hormones, diet, etc. Possible immunotherapies of cancer include antibody therapy, which involves recognizing tumor antigen by antibodies and thus signals the immune system to kill the tumor cells. Cytokine therapies involve the modulations by interferon and interleukin proteins to provoke the immune response to cancer cells. Moreover, combination immunotherapies such as CSF1R inhibitors with TLR agonists can enhance the anti-tumor response, leading to a persistent effect against cancer (Perry CJ et al., 2018).

Leukemia is known as a group of blood cancer that normally develops in the bone marrow, which leads to a large number of abnormal blood cells, and these blood cells are called "blasts" or "leukemia cells" (Leukemia, NCI,1980). However, these abnormal cells would not die in the bone marrow. Instead, they congregate and occupy the healthy cells' space, preventing the normal function of

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lymphocytes eventually. The concrete cause of leukemia is still unsubstantiated (Hutter et al.,2010). Nevertheless, common risk factors may include smoking, benzene exposure, radiation, and family history. Based on the affected cell type, leukemia has been classified into four major subtypes, including acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). Among these four types, myeloid leukemia typically develops in neutrophils or monocytes and mostly happens among adults over 65. AML is usually provoked by the damage of blood cells where the blasts overnumbered the normal functional cells and platelets in the bone marrow, while CML normally occurs due to the translocation of fractions of chromosomes 9 and 22 (Cancer Center, Columbia University). This "swap" may touch off the formation of the oncogene gene BCR-ABL, which secrete proteins to disorder the myeloid environment. Both types may present common symptoms such as anemia and shortness of breath.

On the other hand, lymphocytic leukemia generally engenders B cell, T cell, and NK cells. ALL and CLL's mechanism is similar to that of AML, with rapid growth and domination of blasts in the bone marrow. Diseases involving genetic changes such as AT and down syndrome may induce ALL (Cancer Center, Columbia University), the most common type of leukemia affecting children. In contrast, CLL mainly affects older adults. Anemia, short breath, and appearing pale are shown in most patients affected by lymphocytic leukemia. Potential treatments may include chemotherapy, radiation therapy, targeted therapy, and bone marrow transplantation (NCI, A Snapshot of Leukemia, 2014).

Analogous to most types of cancers, leukemia evolves unique mechanisms to circumvent the regular immune responses in order to survive in the bone marrow. Hence, counteracting these mechanisms because of the specific type of leukemia may help us develop effective immunotherapies. For instance, CD47 corresponding to its ligand SIRPa will be up-regulated in order to avoid HSCs from phagocytosis. AML progenitors, nevertheless, co-opt this mobilization to shirk the recognition by macrophages and thus survive in the bone marrow (Jaiswal et al., 2009) [2]. Aiming at preventing this evasion, scientists have introduced antibodies crosslink CD47, which will induce leukemia cell death (Jaiswal et al., 2009).

Similarly, PU-1 mediated upregulation of CSF1R gene associated with MOZ-TIF2 protein has been verified to be crucial for the survival of AML (Aikawa et al., 2010). Therefore, potential treatment has been detected by ablating the CSF1R expression with AP20187 dimerizer (Aikawa et al., 2010). Other possible immunotherapies focused on leukemia mechanisms have been considered. Synthetic cyclic dinucleotide (CDN) activates the stimulator of interferon genes (STING) pathway and T-cell priming, which secretes cytokines involved in anti-tumor progression (Corrales et al., 2015). Similarly, Chimeric antigen receptor (CAR) T-cell therapy convenes T-cell from the immune system to kill the leukemia cells shows significant outcome, especially in B cell malignancies (Nie et al., 2020) [3]. DC-CIK and NK cell immunotherapies present special effects against ALL and AML as well.

Consequently, based on the immunomodulation mechanisms we know, we raised a hypothesis that combining orthogonal immuno-stimulatory approaches will enhance the anti-tumor effects, and our study speculated several potential combinations and outcomes of leukemia immunotherapies, including the collaboration of anti-CD47/ anti-CSF1R mechanisms, immune checkpoint blockade/ CD40L/ innate immune stimulators, etc. We provided persuasive evidence to support our speculations, and our result may be used as references for potential new treatments in leukemia study. Overall, we believe that immunotherapy's exploitation corresponding to specific kinds of leukemias may obtain breakthrough achievements in anti-cancer studies.

2. Results

In the following section, 6 promising therapeutic approaches for treating leukemia through immunomodulation are discussed, each aiming to enhance anti-tumor, or, more specifically, anti-leukemia effect. Among these therapeutic approaches, some have been tested in clinical trials, while

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others have only been evaluated in preclinical animal models. Although these treatments may be unproven for now, with continuous development in medicine and cell biology, they may shed a different light on leukemia treatments in the future not so far away.

2.1 Anti-CD47 Immune Checkpoint Blockade

CD47 is a protein that is ubiquitously expressed in almost all cells. It can bind to its receptor SIRPα on cell surface of macrophages to inhibit phagocytosis. Hematopoietic stem cells (HSCs) can be mobilized from the bone marrow by the administration of cytokines. While in circulation, HSCs have to pass through the macrophage-lined vasculature of spleen, liver, and marrow in order to approach ectopic niches (Jaiswal et al., 2009) [2]. When the macrophage becomes especially active during inflammation, phagocytosis is very likely to take up stem cells. To protect these stem cells, CD47 binds with its receptor SIRPα, inhibiting the function of phagocytosis by macrophage. That is to say, CD47 performs the function as a "Do not eat me" signal, ensuring that inappropriate phagocytosis does not take place (Buatois et al., Mol Cancer The., 2018). Analogous to hematopoietic stem cells, leukemia stem cells, and progenitor cells also migrate via bloodstream (Jaiswal et al., 2009). Therefore, the phagocytosis mediated by CD47 expression should apply to leukemia as well. While in normal circumstances, CD47 prevents inappropriate phagocytosis from happening, when it comes to leukemia, the leukemia stem cells and progenitors utilize this mechanism to escape phagocytosis. Hence, if we could target CD47 on leukemia progenitor cells, then leukemia, in particular, may be treated (Buatois et al., Mol Cancer Ther., 2018).

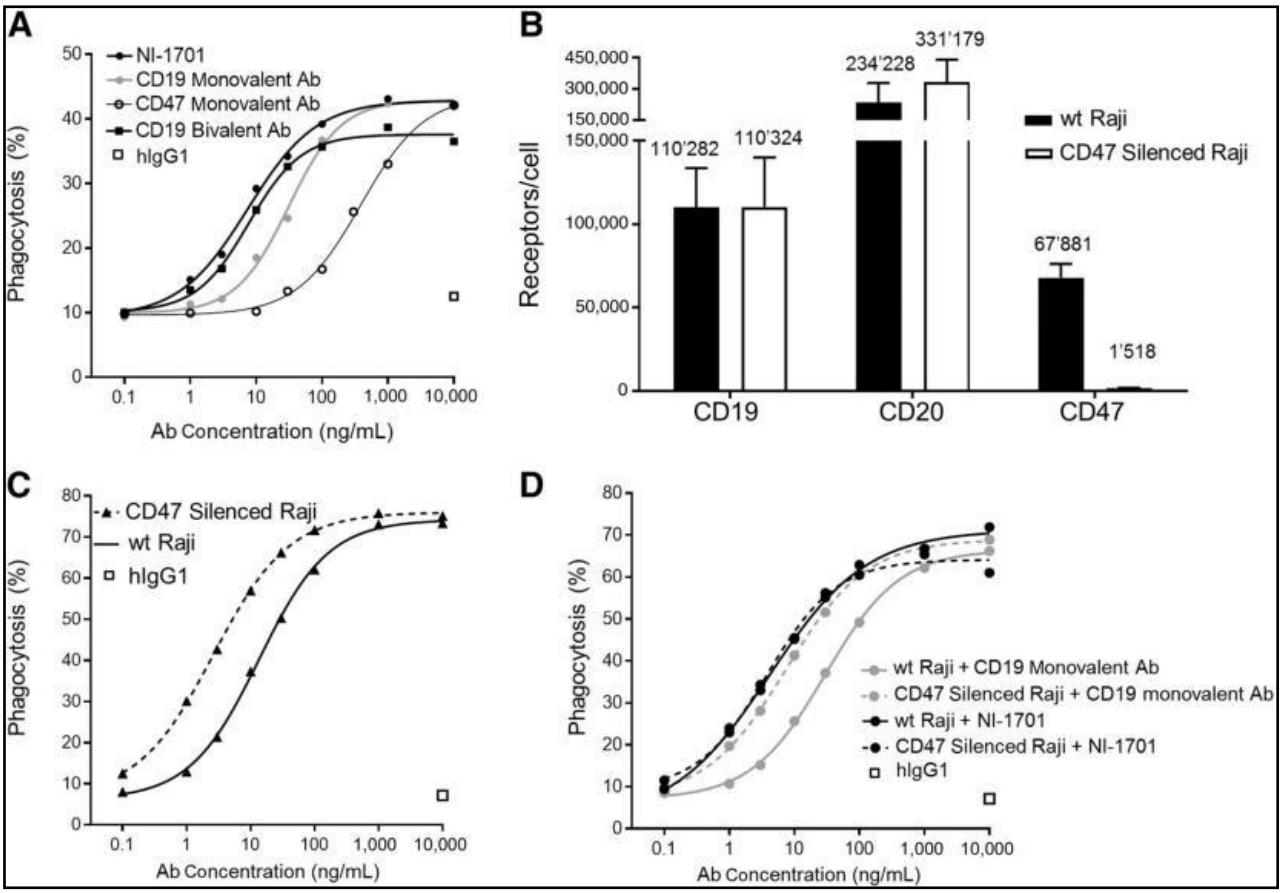

Figure 1 (Buatois et al., Mol Cancer Ther., 2018. [5])

Figure 1 mainly shows the upregulation of phagocytosis induced by CD47 blockade. Panel A portrays the percentage of phagocytosis from treatment with different antibodies. Panel B shows the number of CD19, CD20, and CD47 on Raji cells and CD47 silenced Raji cells. Panel C shows percentage of phagocytosis after rituximab treatment, and Panel D shows the percentage of phagocytosis after NI-1701 or the monovalent CD19 Ab treatment.

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A study conducted by researchers with a fully human bispecific antibody NI-1701 showed that it could co-engage CD47 and CD19 on cells. In figure 1, in the comparison between the percentage of phagocytosis by macrophage with and without the silence of CD47, it is evident that CD47 silenced Raji cells express a relatively higher rate of phagocytosis. Thereby, CD47 is an inhibitory factor for phagocytosis by macrophage, and co-engagement of CD47 and CD19 by NI-1701 can up-regulate phagocytosis in mouse models (Buatois et al., Mol Cancer Ther., 2018). By this mean, if we could target the expression of CD47 on leukemia progenitor cells, then those cells could become targets of phagocytosis and thereby be taken up by macrophage (Jaiswal et al., 2009). NI1701, a bispecific antibody, has been proven to have a more effective anti-tumor function than anti-CD20 targeted antibody; more importantly, when animal models were co-administered with NI-1701 and rituximab (anti-CD20 antibody), synergistic effect is present and results in enhanced tumor regression (Buatois et al., Mol Cancer Ther., 2018). With respect to leukemia treatment targeting CD47, combinatorial therapy, including both NI-1701 and rituximab, is more effective in tumor inhibition than rituximab therapy by itself.

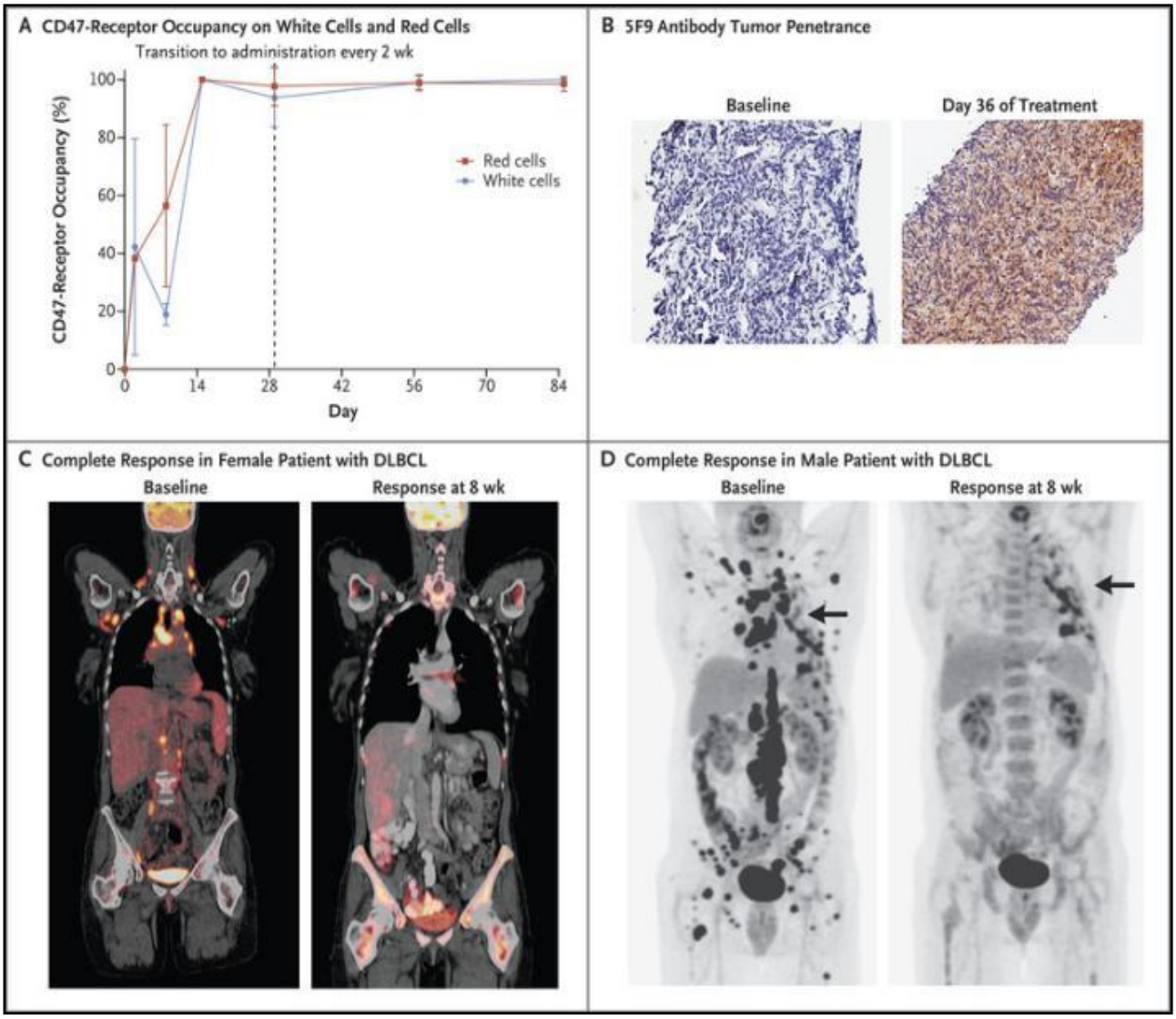

Figure 2 (Advani, et al., N Engl J Med., 2018[4])

Panel A demonstrates the progression of CD47 receptor occupancy on white and red cells. Panel B contrasts tumor 5F9 antibody penetration at baseline and at day 36 of treatment. Panel C exemplifies a complete response of antibody in DLBCL patient, and Panel D shows a complete response in male DLBCL patient. The response is relatively evident.

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In another clinical evaluation of similar CD47 therapy, a group of researchers alternatively use Hu5F9-G4 antibody to inhibit the expression of CD47 and thereby induce phagocytosis. Hu5F9-G4 antibody could synergize with rituximab in order to eliminate B-cell non-Hodgkin's lymphoma cells (Advani et al., N Engl J Med., 2018) [4]. Rituximab by itself is a targeted cancer drug that has an anti-tumor effect; however, its effect is limited toward certain refractory diseases. Together with rituximab, the Hu5F9-mediated phagocytosis is further augmented, as rituximab induces complement and natural killer cell mechanisms.

Moreover, a typical downside of anti-CD47 therapy is that unregulated phagocytosis may take up normal cells beside tumor cells as CD47 is widely present among normal cells, and Hu5F9 antibody, by contrast, enables the selective elimination of malignant cells but not normal cells (Advani et al., N Engl J Med., 2018) [4]. This accounts for a particular advantage of Hu5F9 antibody. In human trials, 22 patients with refractory diffuse large B cell lymphoma (DLBCL) or follicular lymphoma were treated with Hu5F9-G4 antibody in addition to their previous refractoriness toward rituximab. As a result reveals, in figure 2, a complete response in a male patient with DLBCL demonstrates an obvious regression of tumor cells. Among all patients who have been treated, 50% have shown objective responses, and 36% have developed a complete response. Hence, Hu5F9 anti-CD40 has a promising potential in treating tumor cells, but the objective response and side effects are insomnia and dystopia (Advani et al., N Engl J Med., 2018) await to be resolved [4]. However, augmentation of anti-tumor effect by Hu5F9 has largely improved the effectiveness of rituximab treatment with respect to refractory diseases.

2.2 Anti-CSF1R Blockade

Colony-stimulating factor 1 receptor is a cytokine responsible for the survival, differentiation, and proliferation of myeloid-lineage cells (Edwards et al., 2019) [6]. In an acute myeloid leukemia (AML) model, leukemia is induced by the leukemia-associated monocytic leukemia zinc finger (MOZ)-TIF2 fusion protein, which could engage with transcription factor PU.1 and lead to the expression of CSF1R. Also, the study reveals that cells expressing high amounts of CAS1R are likely to initiate leukemia. Thereby, inhibition of CSF1R expression could suppress the induction of MOZ-TIF2 - induced leukemia, achieving cancer stem cell eradication.

The researchers first need to locate CSF1R in order to accomplish an inhibitory blockade. CSF1Rs are located on a subpopulation of supportive cells rather than leukemia blasts. Hence, the design of inhibitors needs to bind with CSF1Rs on the surface of supportive cells in order to decrease CSF1R expression.

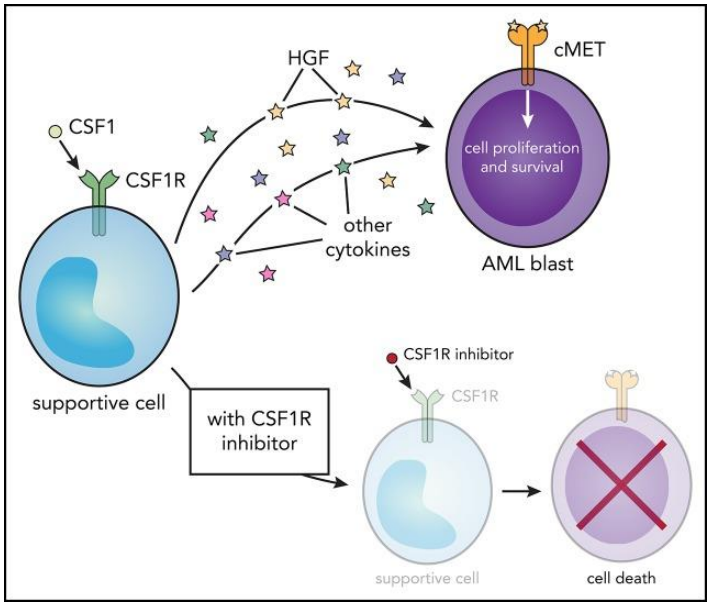

Figure 3: CSF1R is located on a subpopulation of supportive cells (Edwards et al., 2018).[6]

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A clinical trial of CSF1R blockade dose-escalation study for tenosynovial giant-cell tumor is conducted with the generation of a selective CSF1R inhibitor, PLX3397. 14 patients enrolled in an efficacy assessment regarding the volume of tenosynovial giant-cell tumors, among which most patients displayed different extents of tumor regression, and 14 patients have an average 61% decrease in tumor volume score. Meanwhile, adverse effects, including fatigue, change in hair color, nausea, dysgeusia, and periorbital edema, occurred in treatment. The occurrence of these adverse effects resulted in disruption of treatment. Even though the results are not perfect, and patients developed different responses toward CSF1R blockade, overall, this therapy has been proven to have an effective anti-tumor effect. In the context of leukemia, CSF1R blockade has a promising prospect of treating leukemia, particularly acute myeloid leukemia, which theoretically could be cured by the ablation of CSF1R-high cells. Therefore, more studies need to be conducted to overcome potential adverse effects and accomplish accurate targeting of CSF1R in order to cure leukemia.

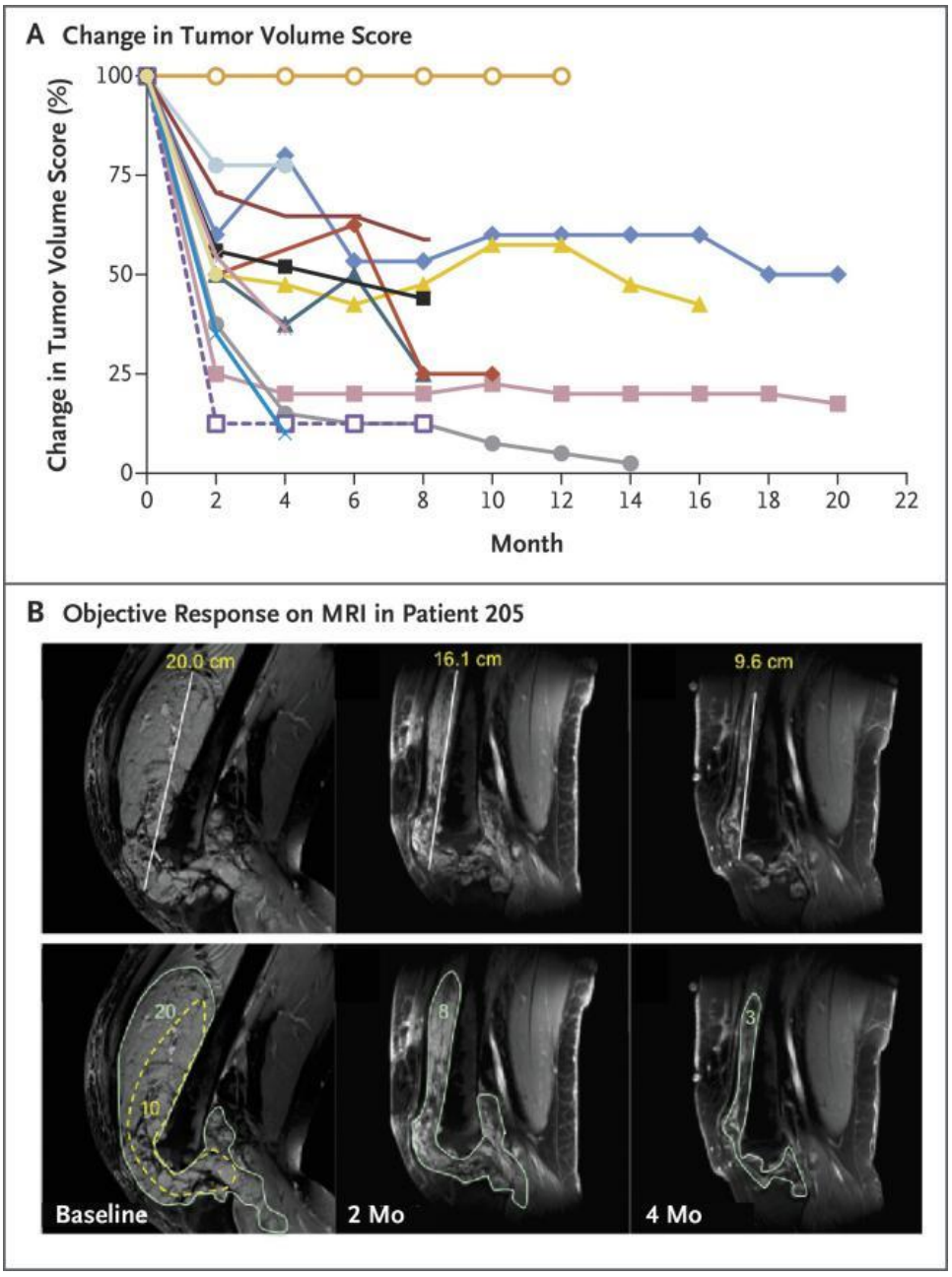

Figure 4 (Tap et al., N Engl J Med., 2015[24])

(A)A line graph lists the progression of change in tumor volume score in patients receiving CSF1R blockade therapy. (B)Objective response observed under MRI. [7]

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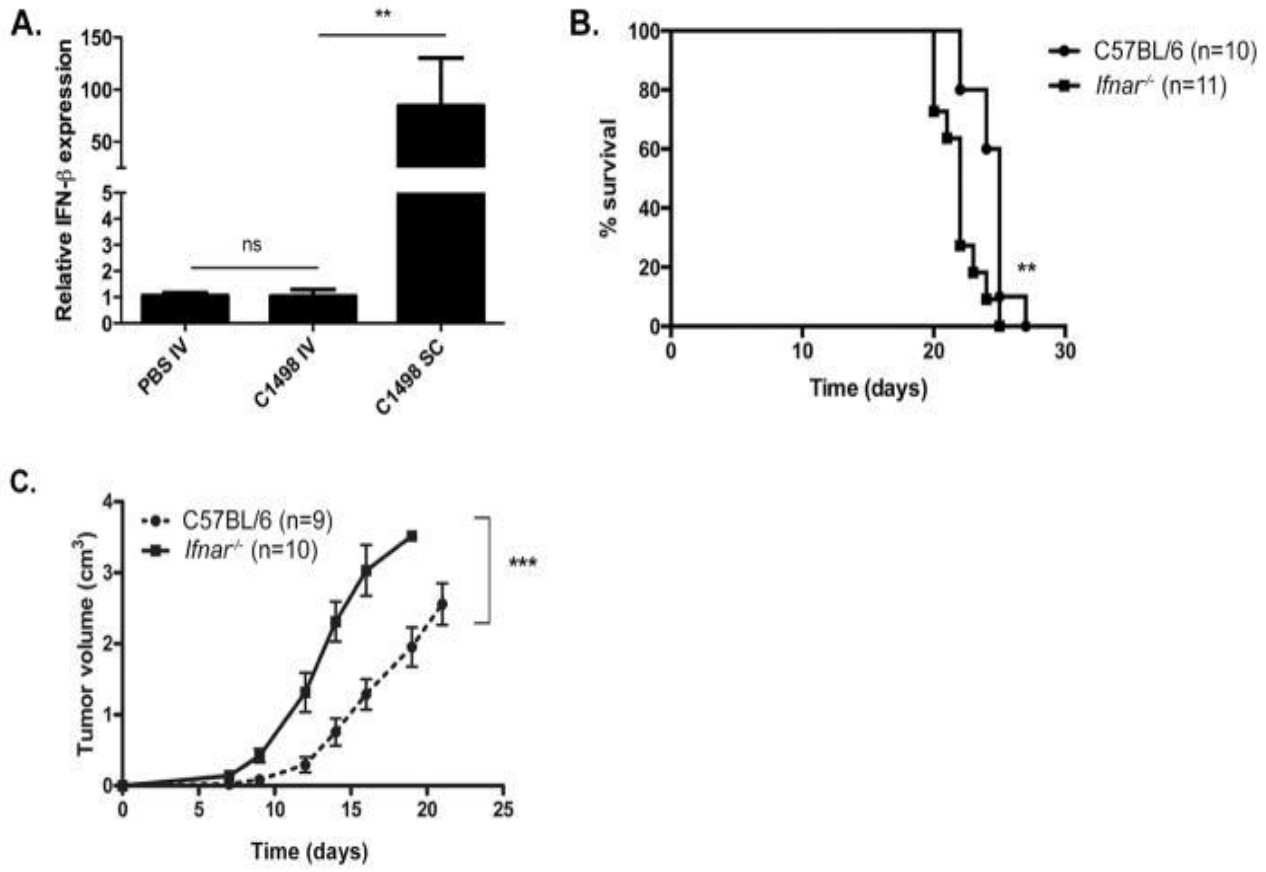

Figure 5 (Curran et al., 2016[7])

(A) Interferon-beta expression is relatively low in mice received PBS IV and C1498 IV inoculation than nice inoculated with C1498 SC; (B) the percentage of survival shows C57BL/6 and Ifnar−/− mice challenged with C1498 cells IV; (C) tumor volume is portrayed for mice received C1498 SC. In conclusion, in AML mice, interferon type I is not expressed [7].

2.3 STING Agonists

The stimulator of interferon genes (STING) is a central mediator of innate immunity in the human body, as it induces the expression of type I interferon and cytokines, which could lead to T cell priming and activation of adaptive immunity. The cGAS-STING pathway can be activated in cells by tumor DNA (Curran et al., 2016), which triggers inflammatory response and recruits immune cells such as B cells and natural killer (NK) cells to fight against cancer cells [7]. However, in animals with disseminated acute myeloid leukemia (AML), it seems like type I interferon is not elicited, and even without type I interferon, the survival of leukemia-bearing animals does not decrease, which indicates that AML may not trigger STING (Curran et al., 2016). In Figure 5, the graph depicts a comparison between mice inoculated with C1498 cells and their PBS-IV wild type counterparts (Curran et al., 2016). This shows that interferon type 1 is not expressed in AML-infected animals. Since AML cannot activate STING, it would not express type I interferon and cytokine, resulting in significant immunosuppression. Thereby, STING activation is essential for restoring immune response against acute myeloid leukemia and treating cancer.

In figure 6, the consequences of lack of type I interferon production and its counterpart, a potential approach to treating AML, is demonstrated. Since STING does not recognize AML, then the production of type I interferon is inhibited, which prevents the following maturation of dendritic cell and T cell priming, resulting in overall poorer survival. On the other hand, if STING can be successfully activated, then type I interferon can be produced, and dendritic cells normally mature, then T cells specific for AML will eliminate some tumor cells and, in return, suppress cancer.

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Another study verified that STING activation also enhances cetuximab mediated NK cell activation and DC maturation. Candidates for STING activation, or STING agonists, are therefore devised.

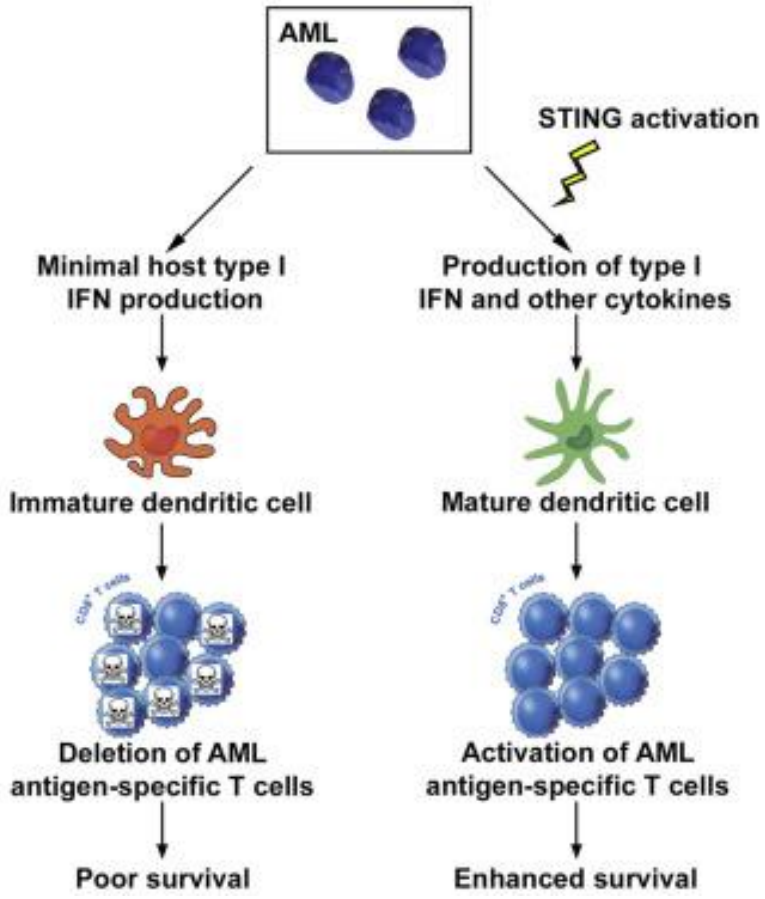

Figure 6 (Curran et al. 2016)

Without STING activation, immature dendritic cells do not lead toward at cell priming, whereas with STING activation, mature dendritic cells activates antigen-specific T cells and enhance survival. [7]

Synthetic cyclic nucleotides and DMXAA are two agonists put on trials and proven to be successful. Spontaneous tumor-initiated T cell priming is dependent on IFN-β production (Corrales et al., 2015). In mouse model, DMXAA induces the expression of Interferon beta in spleen cells, which results in an increase of TNF-alpha and interleukin-6 expression.

Moreover, DMXAA activates host antigen-presenting cells, stimulating leukemia-specific CD8 T cell response. As revealed in figure 7, successful STING activation leads to a much more prolonged survival of mice bearing acute myeloid leukemia.

Upon the utilization of agonists by itself to activate STING, combination therapy involving agonists

Moreover, the immune checkpoint blockade is believed to have more substantial-effectiveness. As a matter of fact, in many cases, mere T cell activation and differentiation are not enough to suppress tumors, as the tumor poorly responds to adoptive T cells. For instance, anti-PD-1/PD-L1 antibody therapies require an ongoing immune response to operate fully. Therefore, without STING

activation leading toward the maturation of CD8 T cells and dendritic cells, the therapy is unlikely to work. Hence, in this case, STING agonists could first be applied to stimulate STING cells, after which CD8 T cells and dendritic cells will proliferate, creating a suitable environment for anti-PD-1/PD-L1 antibody therapy. Thus, neither agonists nor immune checkpoint blockade by itself is as effective as a combination therapy involving both approaches.

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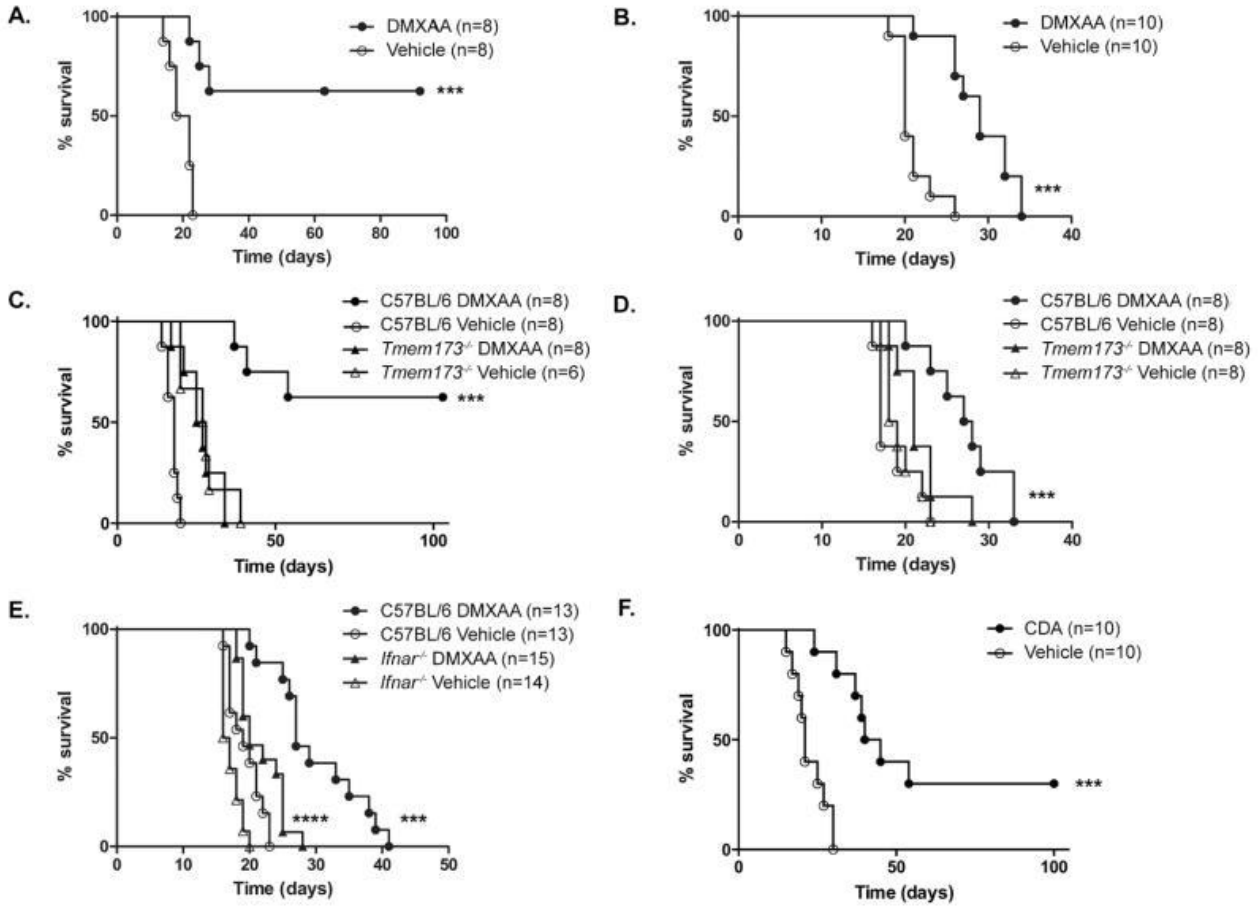

Figure 7 (Curran et al., 2016)

Tumor survival is enhanced by STING activation. C57BL/6, STING deficient(Tmem173−/−), or Ifnar−/− mice are treated with DMXAA or other vehicles, and survival percentage is observed. Prolonged survival is apparent. [7]

2.4 Chimeric Antigen Receptor T-Cell Therapy(treat CLL)

Car-T cell immunotherapy, a relatively new type of cancer immunotherapy, and has drawn considerable attention. The main treatment process and principle feature the extraction of T cells from the patient's own immune system, in vitro culture and modification, equip these T cells with special molecules so that they can recognize and attack specific cancer cells, and then inject the modified T cells back into the patient's body. When the modified cells are returned to the body, they are destroyed by the T cells' immune response to the malignant cells of leukemia.

Significance of CAR-T cell immunotherapy in the treatment of leukemia:(Pan C Y; et al., 2017)

1 [8]. Cll-1 receptors target leukemia stem cells (a small subset of slow-growing leukemia cells that are resistant to conventional drug therapy and can proliferate steadily into new leukemia branches);

2. CD33 receptor targets most AML cells;

One of the keys to CAR-T therapy is to equip the patient's T cells with molecules that recognize specific cancer cells, like special detectors. The modified T cells become CAR-T cells. They move around in the body, and when the detector picks up a specific signal from the surface of another cell, it activates the CAR-T cells and attacks, killing the cell with the signal as an enemy.

A clinical trial has reported mature results from a pilot trial of 14 patients with relapsed refractory CLL treated with CAR-T cells. Autologous T cells (CTL019) from CD19 CAR were introduced with lentiviral vector and injected into patients with recurrent/refractory CLL at doses of 0.14×108 to 11×108 CTL019 cells (mean 1.6×108 cells). (Figure.1)Toxicity, response, amplification, and

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persistence of CTL019 in patients were monitored. Among the CLL patients treated multiple times, the total response rate was 8/14(57%), with 4 patients in complete response (CR) and 4 patients in partial response (PR). The expansion of CAR T cells in vivo is related to the clinical response. In the first two patients who received CR, CAR T cells' function in vivo was maintained for more than 4 years. None of the CR patients had a recurrence of the disease. Patients who received CR and did not relapse averaged 40 months (range, 21 to 53 months, calculated from the infusion). Remission included removing minimal residual lesions (MRD) and evaluating subjects 01, 02, 09 at month 1 and 10 at month 3 with no CLL clones found in their blood and bone marrow. All of the responders had B-cell regeneration disorders and cytokine release syndrome associated with CAR-T cell amplification[9]. Minimal residual lesions were not detected in patients who received CR, suggesting that a cure for advanced CLL is possible[9]. (David M. 2015)

2.5 DC-CIK biological cell immune therapy (treat ALL)

Dendritic cells (DCS) are powerful antigen-presenting cells that engulf, process tumor antigens, and deliver antigenic signals to lymphocytes.

CIK cells (cytokine-induced killer) are a group of heterogeneous cells obtained after a period of co-culture of human peripheral blood mononuclear cells in vitro with various cytokines (such as anti-CD3MCAB, IL-2, IFN-, IL-1A, etc.) [10].(CONG CHEN et al., 2018) Since these cells express CD3 and CD56 membrane protein molecules simultaneously, they are also called NK cells (natural killer cells) like T lymphocytes, with the strong anti-tumor activity of T lymphocytes and the non-MHC (major histocompatibility complex) restrictive anti-cancer activity of NK cells[10]. CIK cells are non-MHC-restricted tumor cytotoxic T cells and are the most active immune effector cells known. CIK cells' application is considered the first choice for new - substitute anti-tumor adoptive cellular immunotherapy. DC and CIK are two important parts of tumor immunotherapy. The former recognizes pathogens and activates the acquired immune system, while the latter kills tumor cells by playing autocytotoxicity and secreting cytokines, which together ensure the completion of a highly efficient and harmonious immune response. DC and CIK cells have certain complementary effects in anti-tumor cells. Combined application will achieve "1+1> 2 ". DC-CIK immune cell method is to induce the differentiation of dendritic cells in vitro, and then use antigen-stimulated dendritic cells to induce CIK cells to produce a specific tumor-killing effect.

Medical research at home and abroad has confirmed that "DC-CIK tumor biotherapy" will become one of the most advantageous and promising biological technologies. It treats tumors on the principle of "killing tumor cells with human body's own immune cells", and at the same time, can enhance the immune function of human body and inhibit the growth of tumor cells. Compared to traditional surgery, radiotherapy, and chemotherapy, biological therapy has incomparable advantages:

1. Safety: Use the body's own immune cells to kill tumor cells without toxic side effects:

2. Pertinence: DC cell recognition, direct phagocytosis of tumor cells, CIK cell-specific killing of tumor cells;

3. Persistence: activate the body's immune system, restore the body's immune function, and kill tumor cells for a long time;

4. Systemic: to rebuild and improve the immunity of patients, identify, search and kill tumor cells comprehensively, and effectively prevent the recurrence and metastasis of tumors;

5. Thoroughness: improve the immune ability of the body, and completely eliminate residual and killing tumor cells and micro-metastasis in the body;

The patient, Mr. Wu, was diagnosed as acute myelogenous leukemia in 2014 at the First Affiliated Hospital of Soochow University. AAG (methotrexate, cytarabine, doxorubicin) was then administered for induction chemotherapy. The end of chemotherapy (January 3, 2015): The blood image gradually recovered. The period of granule deficiency and pulmonary infection was controlled. At that time, the patient's blood image was WBC7.5x109 /L and PLT 24.5x109/L.

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However, continuous chemotherapy has made the patients insensitive to chemotherapy drugs, and only DC-CIK cell transfusion can maintain the remission of the disease if the chemotherapy drugs cannot be continued. However, the effect of DC-CIK continued maintenance treatment was ideal: on January 24, 18, the follow-up condition was basically stable, I felt good, and I had gone to work. Molecular examination (QUANTITATIVE RT-PCR) on March 4, 18: No AML/ETO fusion gene transcriptase was detected. Review on April 15, 18. Bone marrow imaging combined with clinical findings indicated that acute myelogenous leukemia M2 type was in remission, and the patient had completed 8 courses of DC-CIK cell transfusion so far. From October 2014 to May 2013, the survival rate was more than 43 months. The DC-CIK cells survived for 32 months from November 26, 2015, when they were transplanted back.

This suggests the necessity of using DC-CIK cells after chemotherapy-induced remission in leukemia patients: They can not only improve the patient's symptoms and maintain a longer period of remission but also continue to induce remission even in the case of chemotherapy failure. This greatly prolongs the survival time of patients and improves their quality of life. The results show that DC-CIK cell immunotransfusion plays an indispensable role in the comprehensive treatment of leukemia.

In recent years, a large number of in vitro studies have shown that co-cultured DC-CIK cells have highly effective anti-leukemia activity, and the survival time of patients is significantly prolonged, so it has become a research hotspot in the biological immunotherapy of leukemia. Clinical trials showed that after treatment with DC-CIK, the levels of IL-2, IL-12, IL-17, TNF- and IFN- were increased, while the levels of IL-8 were decreased, with statistically significant differences. Wilm's tumor gene is a tumor suppressor gene in WT1 on 11P13. The normal expression can be seen in kidney, testicular support cells, decidual cells, ovarian granulosa cells, and some hematopoietic cells. Wilm's tumor, mesothelioma, and some leukemias can be positive. WT-1 tests of small residual lesions before and after treatment showed that WT-1 was positive in 2 AML patients before treatment, turned negative after treatment with DC-CIK, and remained negative for more than 9 months. The levels of IL-17 were lower in those with remission after chemotherapy and in those with bone marrow suppression after chemotherapy, both of which were lower than those of healthy controls. Treatment with DC-CIK regimen was close to the normal control group. These results suggest that DC-CIK can be used as an important method to eliminate residual leukemia and make it possible to cure leukemia, thus providing a theoretical basis and clinical significance for cell immunotherapy to eliminate residual leukemia. (Rafaela G.A. Costa et al.)

2.6 CAR-NK cell immunotherapy(treat AML)

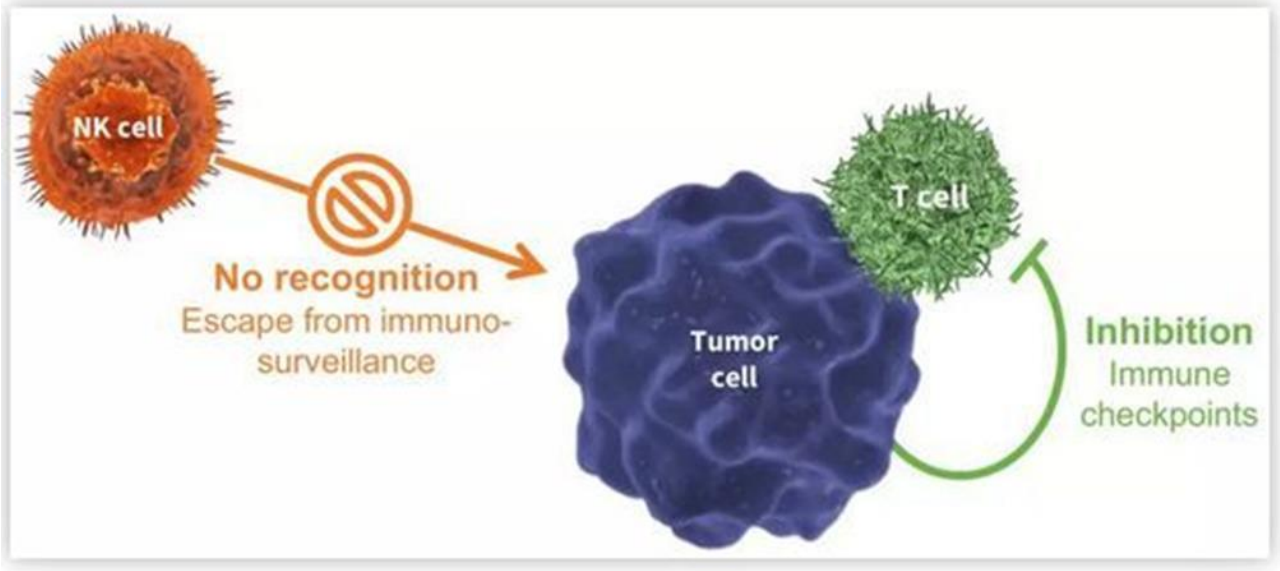

Figure.8 (& Sandro Matosevic 2020)Schematic of NK cell escape [11]

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The specific method of NK cell immunotherapy is to extract immature immune cells from the patient (blood collection), conduct activated culture in the laboratory, and make them have the ability to recognize and kill tumor cells effectively, and then inject them back into the patient. It can break the body's immune tolerance, activate and enhance the immunity of the body, and has the dual effect of treating and preventing tumor. Moreover, patients only need to cooperate to do blood collection and transfusion two steps, do not need to be hospitalized.

NK cells, as an immune weapon to kill tumor cells, need to find tumor cells to function. There is growing evidence that some cancers can evolve multiple strategies to evade recognition by CD8+T cells. Similarly, NK cells are often confused and unable to find tumor cells, and subtle tumors have also evolved mechanisms to evade NK cell surveillance and impair NK cell recognition. For example, the stress proteins expressed on the surface of tumor cells are the recognition markers of NK cells, but tumor cells will escape the immune system's surveillance by shedding proteins, mainly MICA and MICB. (Figure.8)

So to stop this immune escape and allow NK cells to recognize tumors effectively, an antibody targeting MICA and MICB are needed to increase the levels of MICA and MICB on the surface of the cancer cells. (Figure.9)

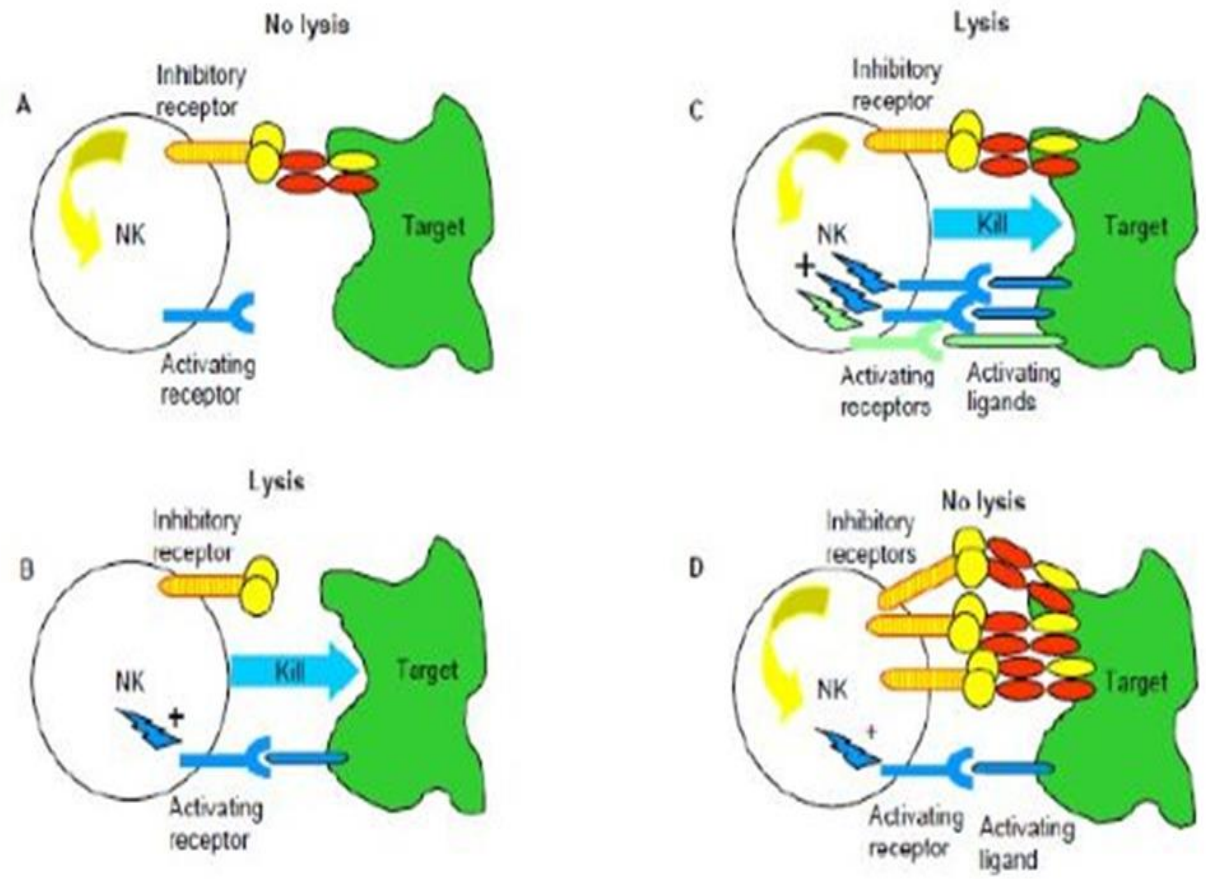

Figure.9 (Luangtrakool Panpimon et al., 2020) How to target MICA and MICB antibodies to increase MICA and MICB levels on the surface of cancer cells [12]

In addition to directly killing ordinary cancer cells, NK cells also have the property of killing tumor stem cells, so as to inhibit the growth and spread of tumors and effectively prevent the recurrence and metastasis of tumors. In general, NK cells have the advantages of high quantity, high purity, high activity, and strong killing activity.(Figure.10&Figure.11)

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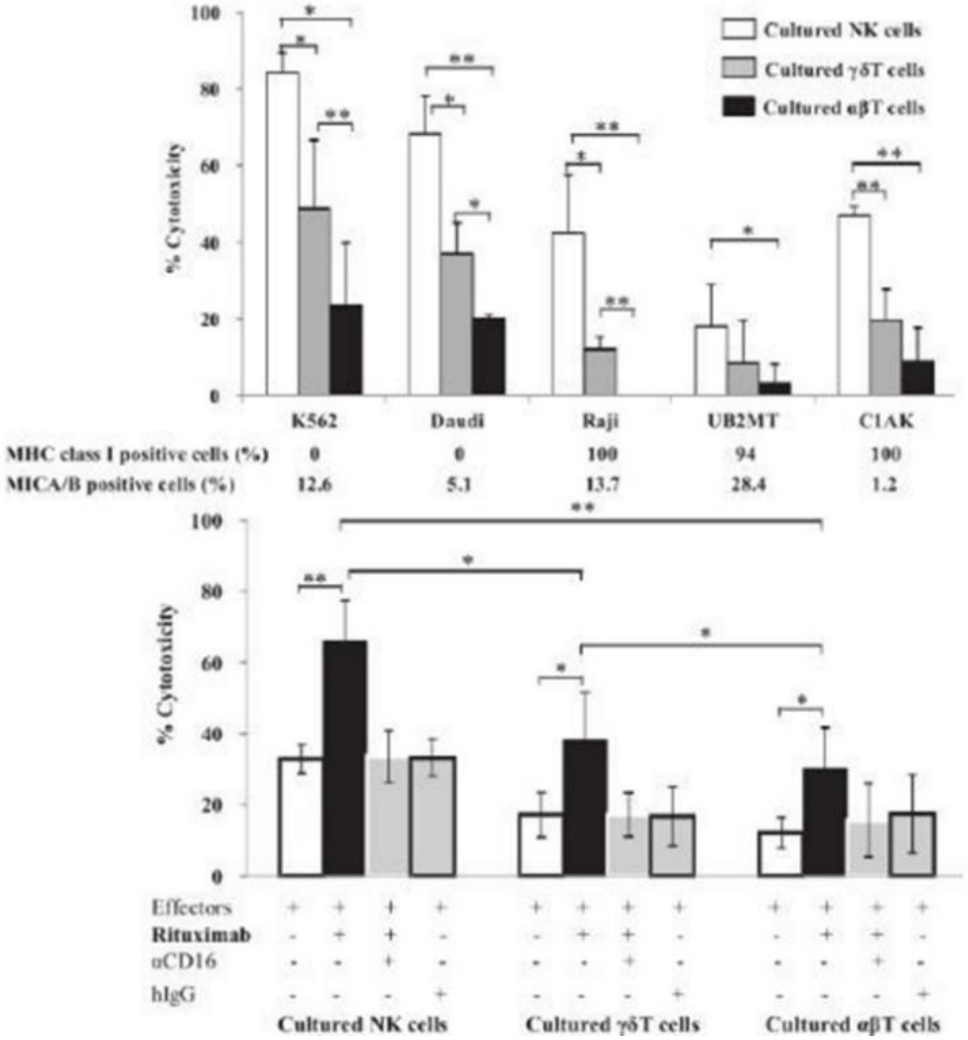

Figure.10 Specificity of NK cells (Yang Yuhui Cancer Center, Union Hospital et al) [13]

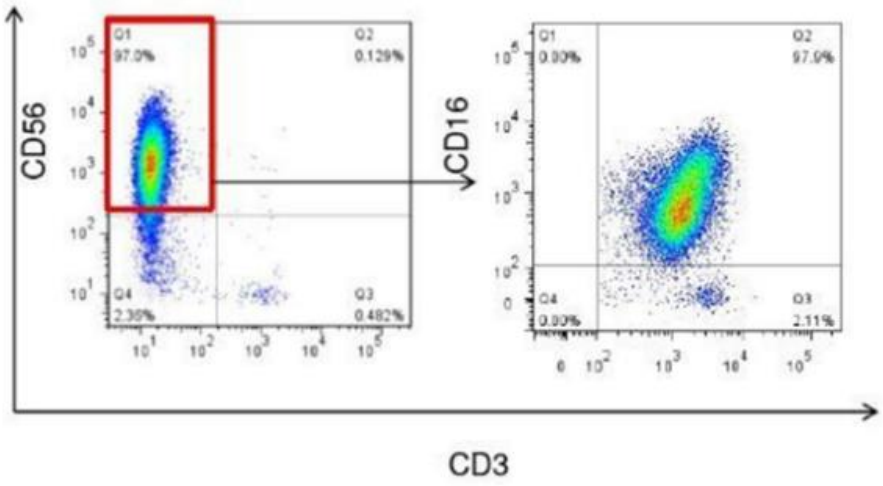

Figure.11 (Childs Richard W et al., 2015) NK cells have the advantages of high quantity, high purity, high activity and strong killing activity. [14]

Based on the GVL triggered by KIRs Mismatch, NK cells were the primary effector. In a clinical study of 112 patients at high risk for AML, the NK cell Alloreaction triggered by the Mismatch between the donor and the recipient was associated with a good prognosis. (Figure.12)

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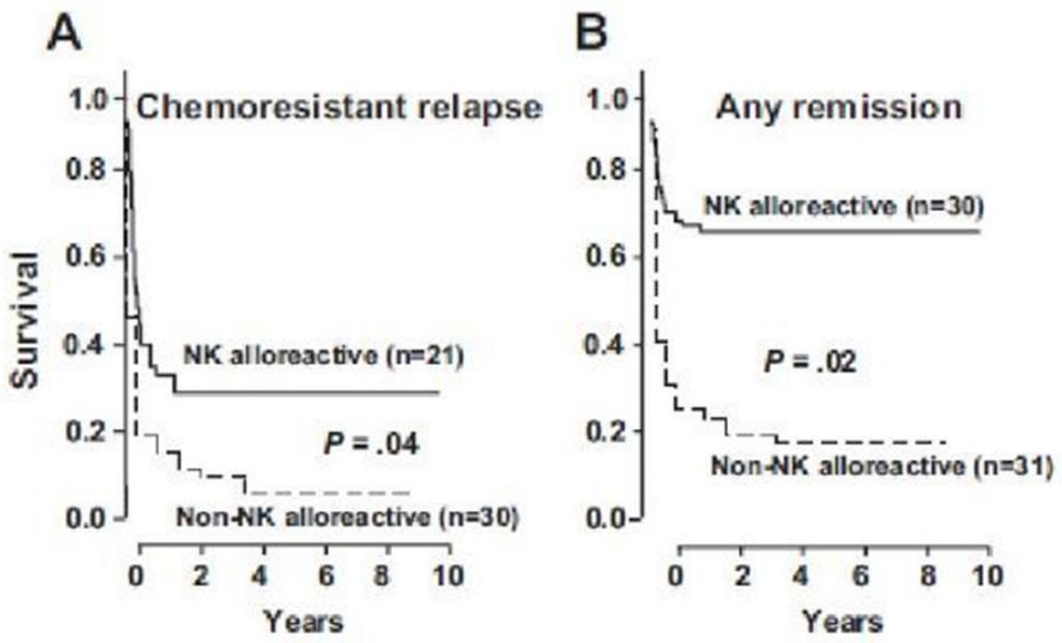

Figure.12 (Kumazaki H et al., 1996) Transplantation from haplodentical NK alloreactive donors improves EFS [15] (A) EFS in patients transplanted in relapse from NK-alloreactive versus non-NK alloreactive donors. (B) EFS in patients transplanted in CR from NK alloreactive versus non-NK alloreactive donors.

In March 2020, researchers at the University of Texas MD. Anderson Cancer Center in the United States came away with good news: The results of a clinical trial of CAR-NK cells showed that most patients with NKL lymphoma and CLL leukemia responded to car-NK therapy. More encouragingly, there were no serious side effects. To some extent, this means that some patients can use immune cell therapy to check and control cancer cells. For patients suffering from the pain of chemotherapy and radiotherapy, cellular immunotherapy can extend their lives and improve their quality of life. Of course, neither CAR NK nor other cells can eliminate all tumor cells by single target immune cells, and the long preparation time and high medical cost of fresh NK cells are not a small challenge for patients. I believe that with the continuous development of medicine, with the joint efforts of scientists, all the problems will be solved. We will eventually solve the same as smallpox cancer[16]. (Wenxiu Wang & Changping Wu, 2020)

3. Discussion

Our results show six immunotherapies on leukemia treatment, including anti-47, anti-CSF1R, STING agonists, CAR-T, DC-CIK, and NK cell immunotherapy. These therapies are not specific to leukemia because different types of leukemia have similar mechanisms. Anti-47 antibody and type I anti-CD20 antibody-rituximab combined therapy have a better anti-tumor effect compare to single anti-CD47 antibody or anti-CD20 antibody therapy. Anti-CSF1R antibody can also use in treating another type of leukemia-CLL. Anti-CSF1R antibody and type II anti-CD20 antibody, A101 (Mössner et al., 2010) [17], combinatorial treatment had a more significant effect on slowing tumor than using anti-CSF1R antibody individually in CLL patients and CLL transplanted mice model (Galletti et al., 2016) [18]. Combination of STING activation therapy and anti-PD-1 antibody therapy also has higher efficacy. It had been proved that anti-PD-1 antibody combined with DC-CIK immune cells could have a synergistic anti-tumor effect for treating renal carcinoma (Yuan et al., 2019) [19]. Maybe we can also utilize this combination to suppress leukemia. In conclusion, we can combine different immunotherapies that target different immune mechanisms such as immune activation and immune checkpoint blockade to treat tumors, and combinatorial tumor-targeting approaches tend to have better tumor-suppressive effects than using either one alone.

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The immunotherapies that we can combine are those that have a certain connection with each other so that there will be an enhanced anti-tumor effect. For instance, the activation of STING pathway can lead to T cells' maturation and dendritic cells, which then allows the anti-PD-1 antibody to have a continuous lymphocyte response. Since the therapeutic effects of CD47 blockade depend on CD8+ T cells, and anti-PD-1 antibody can enhance the killing effect of T cells on tumor, so we can predict that the combination of CD47 blockade and immune checkpoint blockade will probably increase anti-tumor effective. In addition, the SYING agonists treatment can promote the efficacy of anti-PD-1 therapy, so the combination of these three treatments may have synergistic effects on leukemia. PD-1 expression can be increased by the loss of neoantigen expression, immunosuppressive cytokine signaling, and genomic instability; ultimately, this results in T-cell therapy exhaustion (Wedekind et al., 2018) [20]. PD-1 blockade could lead to T-cell proliferation. PD-1 blockade therapy can enhance the anti-tumor effect of CAR-T therapy. Combined immunotherapy offers a promising prospect for more effective treatment of leukemia.

However, immunotherapy has a lot of side effects, so a combination of different immunotherapy may have more serious side effects. CD47 does not only exist on the tumor cell but on the normal tissue. Even though normal cells lack the "eat me" signal, if a patient is receiving radiation or chemotherapy simultaneously, these normal cells may up-regulate the "eat me" signal and cause the normal cells to be engulfed. Anemia is one of the adverse effects. The immune checkpoint blockade can cause disinhibition of T-cell function, and the activation of T cell produces overmuch inflammatory cytokines, which lead to a series of inflammatory side effects (Postow et al., 2018) [21]. CAR-Ts can lead to rapid immune activation, which causes cytokine release syndrome(Frey et al., 2019) [22]. Without a suitable target for CAR T cells, CAR T cells will likely attack normal cells leading to autoimmune disease. There is a similar condition with NK cell immunotherapy. With all these side effects, if we combine two or more immunotherapies, there may be more severe adverse reactions to the patient, even though that may have a better tumor suppression effect. If we use combination immunotherapy, we should take these conditions into account.

Although immunotherapy has a well anti-tumor effect, immunotherapy is confronted with a challenge. For the CAR-T and NK cell immunotherapy, optimal targets are extremely rare, as most targets expressed on tumors are also expressed on vital normal tissues. The target must be highly expressed on the tumor tissue and poorly expressed on normal tissue so that it can have an accurate therapeutic effect(Cheever et al., 2009) [23].

4. Conclusion

In general, immuno-modulation approaches have been proven to affect treating different types of leukemia substantially. Immune checkpoint blockade, such as antibodies against CD47 and CSF1R, have been attempted earlier. While limiting the expression CD47 can target CD47-mediated phagocytosis against leukemia progenitor cells or leukemia stem cells, anti-CSF1R therapy could reduce the number of cells expressing high levels of CSF1R, which might prevent leukemia progenitor cells from occurring. Moreover, using STING agonists to activate STING signaling pathway ensures that type I interferon is produced properly so that dendritic cells and further adaptive immune response could develop smoothly. On the other hand, CAR-T cell immunotherapy involves the extraction of T-cells from the patients' bodies, which can then be modified with special molecules so that they can recognize and kill cancer cells. Another cellular approach, DC-CIK biological cell immune therapy, consisting of both CD3 and CD56 membrane protein molecules, have a strong anti-tumor effect, making it a potential candidate of anti-tumor cell not restricted by MHC molecules. DC-CIK can be particularly effective in treating ALL. CAR-NK cell immunotherapy, for treating AML, can activate and enhance the body's immunity while preventing tumors. Notably, for almost all of these cancer treatments, the result is evidently more evident when one therapy is combined with another approach. For instance, STING agonist approach yielded a much better result when combined with rituximab treatment in the patient. Because of differences in the DNA of individual patients, immunotherapy drugs can work extremely well in some patients but have little effect on others. Hence,

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combinatorial immunotherapies provide us with a direction to solve these problems and optimize solutions according to the patient's specificity. Since tumor cells also evolve, this provides more options for immunotherapy and, at the same time, makes immunotherapy more challenging. In general, the combination of complementary immunotherapies offers more effective cancer treatment than using a single one alone. However, according to the data in clinical trials, undoubtedly, these immunotherapies still have a long way to go, and the remaining question, such as rejection reaction, still needs to be solved, but with current progress, these approaches have shredded light on contemporary. Therefore, it is reasonable to believe that all the doubts and concerns will be conquered in the future, not so far away.

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Everyone's contribution to the group paper is the same. The ranking is in Original submission order.

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