# Overcoming Barriers to Immunotherapy Treatment in AML

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Abstract

Acute Myeloid Leukemia (AML) is an aggressive cancer with poor prognosis and limited therapies. In particular, it has met with numerous challenges in the development of promising chimeric antigen receptor (CAR) T cell therapy and other emerging immunotherapies. Current standard of care has a high rate of resistance and relapse. The most significant challenge with CAR T cells is finding a suitable antigen target for AML. Several antigen targets including CD33, CD123, CD7, CLL1, FLT3 have been considered and tested in clinical trials but also have potential problems such as on-target off-tumor toxicity. Additionally, new technologies such as SynNotch CARs, the inducible caspase 9 suicide gene, and bi-specific T cell/NK cell engagers are being introduced to ensure more specific and/or complete eradication of AML.

## 1. Introduction

Acute Myeloid Leukemia (AML) is a rapidly progressing cancer of the myeloid cells that is often diagnosed in patients above the age of 60 (Devillier et al. 2018). AML has a poor prognosis with, generally, the 5-year survival being less than 30% and, in patients over 60, the one-year survival rate is 10-15% (Isidori et al. 2021; Marofi et al. 2021). With such a poor prognosis, it is essential to assess the current treatment available for AML.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the sole curative option that exists in AML, but it is often associated with treatment related adverse events such as graft-versus-host disease (Marofi et al. 2021; Devillier et al. 2022). Further, it has historically proven more effective in preventing relapse in cases of patients who were already in remission but less likely to do so in patients with active disease (Devillier et al. 2022; Zhu et al. 2019). The two-year cumulative incidence of relapse was 47% for patients who underwent allo-HSCT in complete remission and was 64% who underwent allo-HSCT with active disease (Limongello et al. 2021). As a result, these limitations called for a more specific and targeted method of treatment such as the chimeric antigen receptor (CAR) T cell therapy.

CAR T cell therapy involves a receptor engineered to target a tumor associated antigen. This therapy shows promise due to its increased specificity in targeting tumors and has already shown success with B cell acute lymphoblastic leukemia/lymphoma (Limongello et al. 2021). In AML, the necessity of a suitable antigen target that won't result in on-target off-tumor toxicity still requires further research (Haubner et al. 2019). Clinical trials have been

conducted and are continuing to evaluate the current possible antigen targets such as CD33, CD123, CD7, CLL1, FLT3, and more. While some, if not all, of these antigen targets have shown promise in CAR-T cell therapy, there are still side effects that must be considered (Bauer et al. 2019). In this following review, we discuss the current standard of care options along with their challenges, the data and evaluation of certain antigen targets in CAR-T cell therapy, as well as a view of emerging immunotherapies in AML.

## 2. Current Standard of Care (Immunotherapy)

### 2.1 Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a standard of care immunotherapy treatment for acute myeloid leukemia (AML) (Devillier et al. 2022). While being a curative option for AML, allo-HSCT can present a myriad of challenges including high risk of relapse, possibility of a fatal outcome due to treatment-related side effects, as well as scarcity of sufficient matches between donor and patient (Limongello et al. 2021; Devillier et al. 2022). Still, allo-HSCT can present promise for AML. Allo-HSCT has become a curative option with a higher success rate than in the past[ZR2] . This is due to advancements in the transplantation procedure including conditioning regimen, improved prevention through anticipation of graft-versus-host disease and improved prevention of other possible treatment related adverse events (TRAEs). A study documenting the benefits of allo-HSCT for AML in patients aged between 60 and 70 years old in first complete remission found that the transplantation lowers risk of relapse and is necessary for increased chance of relapse-free survival (Devillier et al. 2022). Allo-HSCT was attributed to significantly better rates of 3-year relapse free survival (RFS) and overall survival (OS). RFS increased from 19% to 51% and OS increased from 35% to 56% with allo-HSCT (Devillier et al. 2022).

#### 2.1.1 Challenges Regarding Allogeneic Hematopoietic Stem Cell Transplantation

Even with recent technology, it remains that allo-HSCT has several limitations and side effects. Difficulty in finding a donor with a sufficient match is a significant issue. TRAEs associated with allo-HSCT are also a significant issue with allo-HSCT (Devillier et al. 2022). Graft-versus-host disease (GvHD) is a TRAE in which the graft immune cells see the host body and cells as foreign and results in the graft cells attacking the patient. In a study observing AML patients post allo-HSCT, of 126 cases, 13 developed acute graft-versus-host disease (aGvHD) and seven developed chronic graft-versus-host disease (cGvHD). It was found that grade III-IV acute graft-versus-host disease (aGvHD) was associated with lowered overall survival (hazard ratio: 2.688, $p<0.005$). Two of the patients with aGvHD died from the effects. In general, allo-HSCT presents complications post transplantation. Within the same study, it was

documented that 27 patients died from side effects caused by the transplantation due to immunosuppression. The majority of the deaths were due to infection. (Zhu et al. 2019). A further limitation is that patients were found to do better if they were already in remission prior to undergoing the transplantation. 44.2% of cases were found to relapse after allo-HSCT if they were not in complete remission to begin with (Zhu et al. 2019). This would mean that the patients would have go through another treatment in order to attempt to ensure remission even before allo-HSCT (Zhu et al. 2019; Devillier et al. 2022).

### 2.3 Immune Checkpoint Inhibitors

Immune checkpoint inhibitors (ICIs) were developed to combat immune checkpoints. Tumor cells and other antigen presenting cells can express T cell inhibitory molecules. Immune checkpoints are where those molecules on the tumor cell bind to the T cells and induce apoptosis or exhaustion of the T cell (Daver et al. 2021). PD-L1 and CTLA-4 are molecules that are expressed on AML cells and inhibitory drugs for both have been developed. In particular, ICIs will prevent the tumor cells from causing exhaustion of T cells by means of PD-L1 and CTLA-4 and will thereby allow the T cell to deactivate the tumor cell as per its normal function (Gómez-Llobell et al. 2022). In a phase II trial evaluating azacitidine and nivolumab, a PD-1 inhibiting antibody, 70 patients with refractory/relapsed (R/R) AML were treated with azacitidine. The control consisted of a historical cohort of 172 patients with R/R AML treated by HMA-based clinical trials. The historical controls had received less exposure to HMA-based therapies than those 70 patients in the phase II trial. There was an overall response rate (ORR )of 33% in comparison to the 20% ORR of the historical controls included in the study. Furthermore, four patients (6%) showed complete remission and seven patients (10%) showed hematologic improvement for six or more months (Daver et al. 2019). In a phase II trial in which 38 patients with refractory/relapsed (R/R) AML, 37 of said patients were treated with high-dose cytarabine (HiDAC) followed by administering of pembrolizumab. All patients had received intensive induction chemotherapy as initial treatment and 76% received the treatment on study as the first salvage therapy. The overall composite complete remission (CRc) rate 38% and the median overall survival (OS) was 11.1 months. The overall response rate was 46%.
Common pembrolizumab related toxicities that were seen, most being grade 1/2, were febrile neutropenia (62%), alanine amino transferase elevation (41%), hypocalcemia (30%), alkaline phosphatase elevation (30%), aspartate aminotransferase elevation (30%), hyperbilirubinemia (30%), lung infection (26%), and hypokalemia (24%). Grade ≥3 adverse events included maculopapular rash (5%), aminotransferase elevation (5%), and lymphocytic infiltration on liver biopsy (3%). Grade ≥3 adverse events were rare. There was no treatment related death and 30-day mortality rate was 0% while 60-day mortality was 3%(Zeidner et al. 2021) .

#### 2.3.1 Challenges Regarding Immune Checkpoint Inhibitors

ICIs have shown more efficacy when paired with another treatment but fail to create the necessary effect as a stand-alone treatment (Daver et al. 2021; Gómez-Llobell et al. 2022). In the same phase II trial mentioned above, it was seen that many of the patients had undergone prior treatment or multiple treatments. Specifically, 45 patients (64%) had hypomethylating agent-base (HMA-based) therapy, 27 patients (39%) had high-dose cytarabine (HiDAC) therapy, 21 patients (30%) had intermediate-dose cytarabine (IDAC) therapy, and 33 patients (19%) underwent targeted therapies (Daver et al. 2019). Another issue with ICIs is immune related adverse events (IRAEs). Some common IRAEs are skin rash, pneumonitis, nephritis, and transaminitis (Daver et al. 2019). Of the 70 patients, 11% developed grade III/IV IRAEs. Twenty-four patients treated with the anti-CTLA-4 antibody, ipilimumab, and 25% had grade III/IV immune mediated toxicities (Naval G. Daver et al. 2019; Daver et al. 2021; 2019). ICIs are overall an efficient treatment in overcoming challenges with immune checkpoints and are already widely in use. However, it is important to note that ICIs aren't effective as a single treatment against AML and are administered along with other, possibly more curative, options (Table 1).

Table 1: Current Immunotherapies AML

| **Standard of Care/Immunotherapy** | **Benefits** | **Challenges** | **Clinical Trials** | **References** |
|---|---|---|---|---|
| Allo-HSCT | -lowers risk of relapse | -high cost<br>-finding a donor/match<br>-GvHD<br>-other TRAEs | -currently in US: 7<br>-common combination: fludarabine | (Limongello et al. 2021; Devillier et al. 2022; Zhu et al. 2019; Daver et al. 2021) clinicaltrials.gov; 8/5/22 |
| CAR-T Cell | -ensures increased specificity in targeting | -on-target off-tumor toxicity<br>-finding a suitable antigen target | -currently in US: 11<br>-common combination: fludarabine[ZR9] ; cyclophosphamide | (Marofi et al. 2021; Limongello et al. 2021; Daver et al. 2021) clinicaltrials.gov; 8/5/22 |

| | | | | |
|---|---|---|---|---|
| | | -high cost | | |
| ICI | -boosts T cell activity | -needs to be paired with another treatment<br>-IRAEs | -currently in US: 10<br>-common combination: nivolumab; ipilimumab; azacitidine | (Daver et al. 2021; Gómez-Llobell et al. 2022; Daver et al. 2019; Naval G. Daver et al. 2019) clinicaltrials.gov; 8/5/22 |

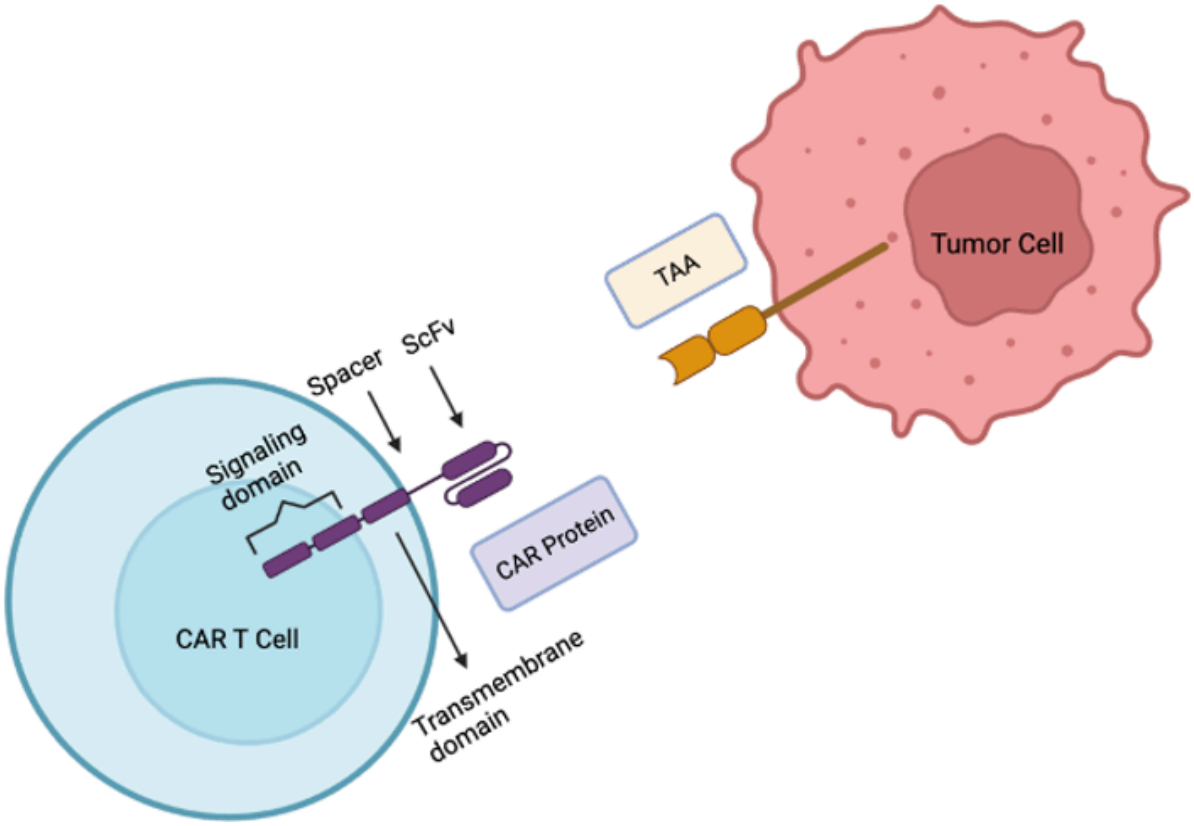
Allo-HSCT: allogeneic hematopoietic stem cell transplantation; CAR: chimeric antigen receptor; ICI: immune checkpoint inhibitor

## 3. Finding An Antigen Target

### 3.1 CAR-T Cell Therapy

Chimeric antigen receptor (CAR) T cell therapy is the use of autologous T cells with genetically engineered antigen receptors to target cancerous cells expressing a specific antigen target.(Figure 1) Specifically for AML, CAR T cells can possibly be engineered to target antigens of a myeloid-lineage which would effectively kill off the myeloid blasts (Gomes-Silva et al. 2019). CAR T cell therapy has found success: especially with CD19 as a target in several B cell cancers like acute lymphoid leukemia (ALL) (Marofi et al. 2021).

Figure 1:




CAR T Cell - Chimeric Antigen Receptor T cells express an engineered receptor that targets a specific tumor antigen. The single-chain variable fragment (ScFv) is derived from the variable region of an antibody that targets the tumor antigen. This is linked to a spacer region followed by a transmembrane domain that allows for flexible targeting on the surface of a T cell. This is then followed by a signaling domain. This receptor is engineered to target a tumor associated antigen. This allows for the CAR T cell to kill the tumor cell after the receptor binds to the targeted antigen.

### 3.1.1 Challenges Regarding CAR-T Cell Therapy

While this therapy has shown promising results in other B cell malignancies, CAR-T cell therapy still has its limitations. The difficulty is that the antigens that are present on tumor cells may also be expressed on normal, healthy cells. The CAR does not have the ability to differentiate between the antigen on the tumor cell versus on the normal cell and will, therefore, target them both (Gomes-Silva et al. 2019). This can lead to an adverse event called on-target off-tumor toxicity. The severity of the issue can vary depending on which category or type of normal cells the target antigen is expressed on. For example, a study done using CD7 as an

antigen target found that CD7 CAR T cell therapy could prove difficult because CD7 is expressed on T cells themselves (Gomes-Silva et al. 2019). Furthermore, while not categorized on-target off-tumor toxicities, cytokine release syndrome (CRS) and tumor lysis syndrome (TLS) are additional treatment related adverse events associated with CAR T cell therapy. Onset of CRS can be characterized by increased levels of cytokines that are associated with systemic inflammatory response. IL-6 and IL-1 are pleiotropic cytokines that are closely associated with the toxicity of CRS and both have pro-inflammatory effects. Symptoms of CRS include hypoxia, hypotension, organ damage, endothelial injury, vascular leakage, cytopenias, coagulopathy, hemophagocytic lymphohistiocytosis (Brudno and Kochenderfer 2019; Morris et al. 2022). In a CD38 CAR T cell therapy trial conducted for patients with relapsed AML post allogeneic hematopoietic stem cell transplantation (allo-HSCT), all six patients enrolled in the trial developed CRS. Five patients had grade I-II CRS and one patient had grade III CRS. Although, these cases were deemed to be clinically manageable throughout the trial (Cui et al. 2021). Additionally, in a CAR T cell therapy study done with T cells co-expressing an anti-CLL1 [ZR10] CAR as well as interleukin-15 (IL15), there was found to be a severe form of CRS in the AML models. In the same study, when the CAR T cells were administered to mice, all the mice that received the therapy developed a syndrome associated with rapid tumor destruction or tumor lysis syndrome. This proved to result in the symptoms of hypothermia, tachypnea, tachycardia, and death (Ataca Atilla et al. 2020).

### 3.2 Defining A Suitable Target

There are three different types of categories of antigens. Leukemia associated antigens (LAAs) are antigens often present on many cancerous cells, but are also expressed on normal cells. Lineage restricted antigens (LRAs) are antigens that restricted by myeloid lineage as how CD33 is restricted to myeloid cells including myeloid progenitor cells, monocytes, and mast cells. Both leukemia specific antigens (LSAs) and neoantigens are more specific to cancerous cells. Neoantigens are antigens that develop as a result of mutations with the cancer. Because they are newly developed as the cancer progresses, they are specific to the cancer and easier to target. However, a challenge is that because mutations vary between patients, the neoantigen will also vary between the patients (Daver et al. 2021). (Table 2). Certain conditions need to be met to find a suitable antigen target for chimeric antigen receptor (CAR) T cell therapy. The antigen target must be expressed on a majority of the tumor cells in order to eradicate most, if not all, of the cancer. But the antigen’s expression on normal, healthy cells must also be considered as that could result in off-target toxicity (Daver et al. 2021; Gomes-Silva et al. 2019).

Table 2: Classification of Antigens

| Antigen Type | Expression/Characteristics | Example | References |
|---|---|---|---|
| **Leukemia associated antigens (LAAs)** | -expressed on cancerous cells and normal cells | WT1, PRAME | (Daver et al. 2021; Guinn et al., 2007) |
| **Lineage restricted antigens (LRAs)** | -present on myeloid cells | CD33, CD123 | (Daver et al. 2021) |
| **Leukemia specific antigens (LSAs)** | -expressed more specifically on tumor cells<br>-are the result of mutations in the cancer<br>-not always expressed on<br>the cell surface | DEK-CAN fusion protein | (Daver et al. 2021; 2016) |
| **Neoantigens** | -are the result of mutations in the cancer<br>-not always expressed on the cell surface | NPM1, IDH1[ZR11] (both are neoantigens when protein is the isoform of leukemias in which these genes are mutated) | (Daver et al. 2021; Roerden, Nelde, and Walz 2019) |

## 3.3 Targets and Outcomes

### 3.3.1 CD33

CD33 is a transmembrane protein that has 90% expression on AML blasts and is also expressed on leukemic stem cells (LSCs). While this makes it seem like a potential target,

CD33 is also expressed on hematopoietic stem cells (HSCs), myeloid progenitor cells, monocytes, mast cells, Kupffer cells, and microglial cells in the brain (Vago and Gojo 2020; Marofi et al. 2021). Because of CD33's expression on these cells, there is a risk of myelosuppression as a side effect of targeting CD33 positive cells (Vago and Gojo 2020). (Table 3)

### 3.3.2 CD123

CD123 is a IL-3 receptor-a with substantial expression on both LSCs and AML blasts (50%-100%). CD123, unlike CD33, it has minimal expression on HSCs. However, it is also expressed on myeloid progenitor cells, monocytes, basophils, dendritic cells, and respiratory and gastrointestinal epithelial cells. In the same manner as CD33, myelosuppression is a possible challenge for CD123 as an antigen target (Vago and Gojo 2020).

### 3.3.3 CD7

CD7 is a transmembrane glycoprotein that has promising potential as an antigen target, but has different challenged than the two antigens previously discussed. However, a significant problem is that CD7 is expressed on normal T cells themselves. Using CD7 as a target could result in the death of T cells, leading to immunosuppression. To combat this, a study was done is which the CD7 gene was edited in primary activated T cells to produce $CD7^{KO}$ CD7 CAR T cells before expression to limit chances of T cell death. The results showed that the removal of CD7 was present in up to 90% of T cells[ZR12] . Another study tested the usage of pharmacologic inhibitors, ibrutinib and dasatinib, to inhibit the cytotoxic signal produced by CD7 CAR T cells towards other CD7 CAR T cells (Watanabe et al. 2022). The result was that the pharmacologic inhibitors suppressed fratricide, defined here as CD7 CAR T cells targeting and killing other CD7 CAR T cells, whereas the unedited CD7 CAR T cells that were not treated with pharmacologic inhibitors resulted in considerable levels of fratricide (Watanabe et al. 2022). CD7 CAR T cell therapy generally demonstrated increased activity in targeting AML blasts, especially in comparison to normal T cells (Gomes-Silva et al. 2019). This means that CD7 shows potential as an antigen target, considering that it is not expressed on many other normal tissues which would cause an issue. Furthermore, CD7 is expressed in 30% of AML and CD7 expression is generally associated with higher chemoresistance as well as higher risk of relapse post allo-HSCT (Gomes-Silva et al. 2019). CD7 CAR T cell therapy would be effective for this 30% of AML cases.

### 3.3.4 CD371 (CLL1, CLEC2A)

CD371 (CLL1, CLEC2A) is a transmembrane receptor with 77%-100% expression on AML blasts as well as expression on LSCs. The normal tissues that it is expressed on are monocytes, granulocytes, and tissue-resident lung macrophages (Vago and Gojo 2020). Given this, CD371 is a viable option because it has relatively high expression on AML blasts and is not expressed on normal tissues that would cause detrimental harm if targeted. While it is seemingly an ideal target, CD371 is newly being introduced in clinical trials. A recent study with T cells co-expressing anti-CD371 CAR T cells and interleukin 15 (IL15) showed persistent anti-tumor activity in xenograft models of AML. However, the CD371-IL15 CARs resulted in cytokine release syndrome (CRS) which was correlated with high levels of tumor necrosis factor alpha (TNFa). The CRS was subsequently prevented through the use of a TNFa blocking antibody. An inducible safety switch [ZR13] known as inducible caspase-9 was also used. Furthermore, *in vivo*, mice with AML patient derived xenografts (PDX) that received CD371 CAR+iC9-1L15 [ZR14] T cells developed a syndrome associated with rapid tumor destruction. Mice that received just CD371 CAR T cells survived longer and did not develop the previously mention syndrome, but they maintained a higher tumor burden (Ataca Atilla et al. 2020). In another phase I study of CD371 CAR T cells, ten patients with relapsed/refractory AML (R/R AML) were enrolled in the trial and received CD371 CAR T cell therapy. All patients developed CRS and nine patients had grade III-IV agranulocytosis due to therapy. Seven patients did achieve complete response/complete response with incomplete hematologic recovery. However, two patients achieving CRi still died due to chronic agranulocytosis (Jin et al. 2022). Between these two studies, it is evident that while CD371 is an antigen target with potential, there is high risk of various treatment related adverse events. More research and trials are yet to be conducted to overcome the challenges seen with CD371 thus far.

### 3.3.5 FLT3 (CD135)

FLT3 is a type III receptor tyrosine kinase with 70%-100% expression on AML blasts. A study done to assess the effectiveness of FLT3 as an antigen target for AML found that when CAR T cells were cultured with AML cell lines, they displayed strong cytotoxicity against the AML blasts exhibiting the FLT3 antigen. Even when tested for long term activity, the CAR T cells were still effective against the AML cells (Sommer et al. 2020). A challenge, however, may be the fact that the FLT3 antigen is also expressed on HSCs, myeloid progenitors, and neurons. HSCs expressing FLT3 being *targeted* by the CAR T cells could be depleted leading to high toxicity (Vago and Gojo 2020).

Table 3: Antigens in CAR T Cell Therapy for AML

| Antigen Target | Type | AML Blast Expression | Normal Tissue Expression | Clinical Trials | References | Notes |
|---|---|---|---|---|---|---|
| CD33 | Transmembrane protein | 90% | HSCs, myeloid progenitors, monocytes, mast cells, Kupffer cells, microglial brain cells | -currently: 13 | (Marofi et al. 2021; Vago and Gojo 2020) clinicaltrials.gov ; 9/16/23 | -potential death of HSCs -potential death of microglial cells in the brain leading to inflammation -potential of myelosuppression |
| CD123 | IL-3 receptor-a | 50%-100% | Myeloid progenitors, basophils, dendritic cells, respiratory & gastrointestinal epithelial cells | -currently: 6 | (Marofi et al. 2021; Vago and Gojo 2020) clinicaltrials.gov ; 9/16/23 | -potential of myelosuppression |
| CD7 | Transmembrane glycoprotein | 30% | T cells, NK cells | -currently: 5 | (Vago and Gojo 2020; Gomes-Silva et al. 2019; Marofi et al. 2021) clinicaltrials.gov ; 9/16/23 | -potential of T cell fratricide |

| | | | | | | |
|---|---|---|---|---|---|---|
| CD371(CLL1, CLEC2A) | Transmembrane receptor | 77%-100% | Monocytes, granulocytes, tissue-resident lung macrophages | -currently: 5 | (Marofi et al. 2021; Ataca Atilla et al. 2020; Jin et al. 2022; Vago and Gojo 2020) clinicaltrials.gov ; 9/16/23 | -potential result of cytokine release syndrome and tumor lysis syndrome |
| FLT3 (CD135) | Type III receptor tyrosine kinase | 70%-100% | HSCs, myeloid progenitors, neurons | -currently: 3 | (Vago and Gojo 2020; Marofi et al. 2021; Sommer et al. 2020) clinicaltrials.gov ; 9/16/23 | -potential death of HSCs |

HSC: hematopoietic stem cell

## 4. New Technology in Acute Myeloid Leukemia

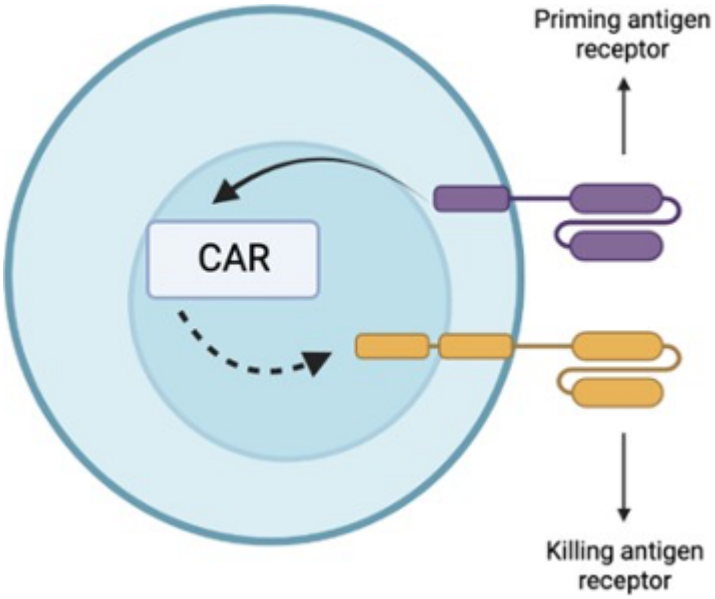
In the face of challenges with the current technologies to combat acute myeloid leukemia (AML), new technologies have emerged that improve the efficacy of cancer eradication. These technologies have the potential fill the gaps of the technologies discussed above.

### 4.1 Synthetic Notch Chimeric Antigen Receptors

Synthetic Notch chimeric antigen receptors (SynNotch CARs) were first described to target solid tumors. (Figure 2) In a study done to kill glioblastomas through the development of SynNotch CARs found that they are ideal because these CARs assume a multi-antigen targeting strategy through multiple receptors. Before SynNotch CARs possible antigens that had been used in clinical trials with regular CARs to combat glioblastomas were EGFRvIII, EphA2, and IL13Ra.
But individually, all three were met with challenges (Choe et al. 2021). The development of

SynNotch CARs included a circuit which first recognize a priming antigen and are then only able to induce the expression of a CAR directed against the killing antigen.

Figure 2:




SynNotch CAR T Cell - Synthetic Notch Chimeric Antigen Receptor T cells are engineered with multiple receptors to recognize separate antigens known as priming and killing antigens. The priming antigen receptor recognizing the corresponding priming antigen and only then does it activate CAR expression. This allows the killing antigen receptor to recognize the killing antigen and kill the cancer cell expressing that antigen.

In this study, the SynNotch receptors were engineered in different ways to test the efficacy of having different priming antigens and killing antigens. The receptor first recognized the cancer specific, heterogeneous EGFRvIII antigen and then targets two homogeneous, less tumor-specific killing antigens, EphA2 and IL13Ra, via CAR expression. This SynNotch CAR receptor was engineered to recognized two killing antigens to further increase chances of complete tumor eradication. Furthermore, a mechanism considered to overcome heterogeneity in this study, known as trans-killing, allowed the SynNotch CAR to even be effective in killing $EGFRvIII^{-}$ cells in vitro. Trans-killing is defined as the process by which a T cell is primed by a cell with the priming antigen but is able to kill a different target that lacks the priming antigen but presents the killing antigen. However, this was only in the presence of priming cells or cells that are $EGFRvIII^{+}$. This was also observed in mice with glioblastoma xenografts. Compared to mice receiving normal T cells, the mice receiving the SynNotch CAR T cells showed significant reduction in tumor growth but only where both the priming and killing antigen were present. In general, further *in vivo* study with mice showed that the SynNotch CAR T cells were able to effectively kill all tumor cells without off-tumor on-target toxicity (Choe et al. 2021). This shows that in the case of solid tumors, SynNotch CARs are

extremely efficient in completely clearing out the tumor. In addition, using SynNotch CARs greatly limit the risk of off-tumor on-target toxicity because of the heightened targeting specificity.

Futhermore, another study done with SynNotch CARs in a solid tumor known as mesothelioma found that effectively targeted two tumor cell lines in vitro compared to the one tumor cell line targeted by normal CAR T cells. In addition, the latter was associated with a slower killing rate of the tumor cells. These same results were found in mice models (Hyrenius-Wittsten et al. 2021). Beyond this, SynNotch CARs have a longer-lived memory and are less prone to exhaustion than normal CAR-T cells (Hyrenius-Wittsten et al. 2021).

While these are both cases of solid tumors, SynNotch CARs can still be adapted to AML. Due to the difficulty of finding a suitable antigen target in AML that doesn't result in on-target off-tumor toxicity, trans-killing could be extremely helpful in that even if the priming antigen isn't present on AML cells while the killing antigen is, employing trans-killing could ensure the death of AML cells with the killing antigen while leaving cells with the priming antigens unaffected. Although trans-killing is less likely to occur in AML since it is not a solid tumor, the multi-antigen targeting mechanism can still decrease the levels of on-target off-tumor toxicity in the situation that the priming antigen is a leukemia specific antigen. This would mean increased specificity of the CAR.[ZR15]

### 4.2 Inducible Caspase 9 Suicide Gene

The inducible caspase 9 (iC9) suicide gene is increasingly being used as a safety switch to control adverse events in CAR T cells therapy. A study done with CD19 CAR T cell therapy for B cell acute lymphoblastic leukemia (B-ALL) included the iC9 suicide gene to overcome the possibility of inadvertent transduction of leukemic B cells with the CAR construct. In such a case, the iC9 suicide gene is activated through binding to the small biomolecule AP1903 which will induce apoptosis of CAR positive B-leukemia/lymphoma cells. *In vitro*, activation of iC9 in the presence of CAR+ B leukemia/lymphoma cells resulted in the elimination of those cell lines. The same results were noted *in vivo*. In nine out of the ten mice studied, CAR+ B-leukemia/lymphoma cells were completely eliminated (Guercio et al. 2021). This empirically proves that the iC9 suicide gene is efficient in controlling adverse events, particularly inadvertent transduction of leukemic B cells with the CAR construct and can be used in CAR T cell therapy in the future.

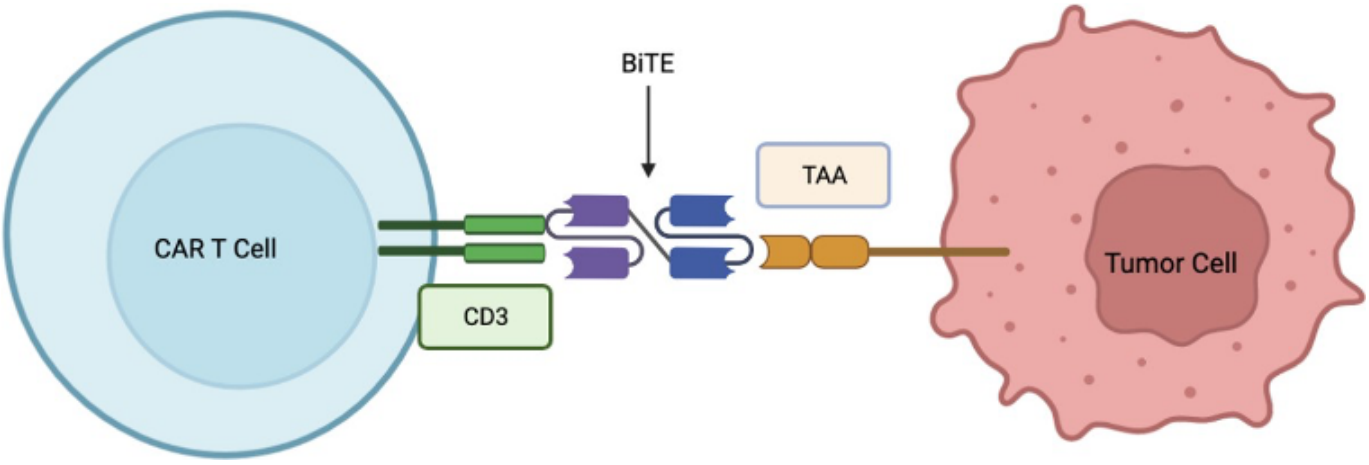
### 4.3 Bispecific T-cell Engagers, Bispecific Killer Engager Antibodies, and Trispecific Killer Engager Antibodies

Bispecific antibodies are molecules engineered with two antigen receptors: CD3 on T cells and a leukemia associated antigens. (Figure 3) This technology, similar to the Synthetic Notch CARs, overcomes the challenge of surface antigen downregulation on tumor cells leading to tumor evasion. Pertaining to the function of bispecific T-cell engagers (BiTEs), once CD3 on the T cell and the surface antigen on the tumor cell both bind to the antibody, the T cell is activated and sends a cytotoxic response to the tumor cell, inducing apoptosis. (Allen, Zeidan, and Bewersdorf 2021). Furthermore, bispecific killer engager antibodies (BiKEs) and trispecific killer engager antibodies (TriKEs) use natural killer (NK) cells and the CD16 receptor found on them.

A BiKE used in AML targeting CD16 and CD33 on tumor cells was able to cause the activation of NK cells and eradicate the tumor cells. TriKEs, which include a third part that contribute to the expansion of NK cell response, were used with CLEC12A and successfully eradicated AML cells in vitro and in mouse models while avoiding death of hematopoietic stem cells (HSCs) (Allen, Zeidan, and Bewersdorf 2021).

These technologies show two reasons why they may be superior to SynNotch CARs. First, in the case of bivalent engagers, both antigens must be expressed on the same cell and do not require expression on other cells in a microenvironment. Second, as soluble proteins, they are better adapted to blood cancers versus solid tumors[ZR16] .

Figure 3:




BiTE - Bispecific T Cell Engagers are engineered with two antigen receptors, for CD3 and a tumor associated antigen (TAA). The receptor binds to CD3 and the TAA. Then, the T cell sends a cytotoxic signal to the T cell which induces tumor cell apoptosis.

## Conclusion

In the field of immunotherapy regarding acute myeloid leukemia (AML), significant progress has been made in several fields including the novel CAR T cell therapy. There exists the possibility of the strategic use of immunotherapy in AML alongside other existing therapies such as chemotherapy, targeted therapy, radiation, allogeneic hematopoetic stem cell transplantation, to increase the rates of success---instead of just as a stand-alone curative treatment. However, there is a still number of challenges that remain, especially when considering the immune related adverse events and the gap that exists in identifying a suitable antigen target. On-target off-tumor toxicity, adverse events, and the difficulty in finding an antigen target that limits these toxicities are significant barriers that exist in advancing research and success with treatments. Although more research is yet to be done in terms of effectively eradicating cancer cells as well as limiting on-target off-tumor toxicity, much potential has been discovered with antigen targets through clinical trials already. CD7 has shown promise as various methods have already been devised to combat its biggest challenge of T cell fratricide. Furthermore, new technologies are already emerging to fill in those very gaps, advance current treatment, and provide valuable opportunities in AML treatment. Synthetic Notch (SynNotch) CARs especially have shown increased levels of tumor targeting specificity as well as a longer-lived memory and lower levels of exhaustion in comparison to normal T cells. However, further research is warranted in terms of adapting newer technologies such as SynNotch CARs to be effective in AML. More research is also needed in finding a suitable antigen target---or even multiple---for AML CAR T cell therapy.

## Methodology

This review focused on the barriers and current state of immunotherapy treatment in AML using PubMed as the primary database. Search terms included but were not entirely limited to “AML”, “immunotherapy”, “allo-HSCT”, “chimeric antigen receptor T-cells”, “immune checkpoint inhibitors”, “SynNotch CARs”, “inducible caspase 9” “BiTEs”, and “BiKEs.” Articles selected were reviews or clinical trial studies that reported on the efficacy or safety of specific AML immunotherapy treatments. Review articles older than 2016 were not selected and clinical trial studies older than 2017 were not selected. Data extracted from clinical trial studies included number of patients, treatment type, overall outcome, and adverse events observed.

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