Bachelor Thesis

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CHIMERIC ANTIGEN RECEPTOR T CELL THERAPY FOR HEMATOLOGIC AND SOLID CANCERS

Why does CAR T cell therapy work well for B cell malignancies, but not for solid tumors?

Summary

Chimeric antigen receptors have shown dramatic improvements in the survival of patients with hematologic cancer. Clinical trials have shown that CAR T cells are safe and feasible for patients with solid tumors, but have shown limited results. The aim of this thesis is to determine why CAR T cell therapy works well for hematologic malignancies, but not for solid tumors. This has been assessed by looking at the efficacy, persistence and toxicity of CAR T-cells in hematologic and solid cancers. CAR T cells need to overcome additional obstacles in solid tumors. The first obstacle is the lack of specific antigen target for solid tumors. Solid tumors rarely express unique TAA's because of tumor heterogeneity. Secondly, CAR T cells show poor trafficking towards solid tumor sites. A match between adhesins, chemokines and chemokine receptors is needed for effective trafficking, which is often lacking in solid tumors. The third hurdle obstacle is the immunosuppressive tumor environment. The TME makes CAR T cell treatment for solid tumors challenging, as the it impedes the CAR T proliferation and their ability to engage with their target antigen. The last obstacle involves the poor persistence of CAR T cells in solid tumors. Finally, novel strategies that might help overcome the obstacles in solid cancers have been highlighted

Introduction

Stated by the definition, immunotherapy is the treatment or prevention of disease that involves the stimulation, enhancement, suppression or desensitization of the immune system. The immune system keeps track of all the cells and substances in your body, and is able to differentiate between the cells of your body and foreign cells. The immune system will take care of most intruders and malignancies in your body, but has a tough time targeting cancer cells. Cancer cells originate from your own body, which means that these cells are sometimes not different enough to be recognized as foreign. This is not the only reason as the immune system will sometimes recognize the cancer cell, but is not able to generate a sufficient response. Cancer cells can also produce substances that prevent the immune system from recognizing them. Clearly there are limits on the immune system to fight cancer on its own, which is why immunotherapies are administered to help the body's immune system fight the cancer cells

The treatment of hematologic and solid malignancies has changed a lot over the past decades. Mostly because of the introduction of T-cell mediated therapies, which have led to the development of successful approaches like bispecific antibodies, immune checkpoint inhibitors, and chimeric antigen receptor T cells (CAR T cells).(1) CAR T cells are engineered T lymphocytes that express a chimeric antigen receptor. The CAR contains parts of the binding site of an antibody directed against tumor associated antigens (TAAs) (2) Antibody based receptors can recognize pre-determined tumor antigens independent of major-histocompatibility-complex (MHC) restricted presentation. (3) This enables CAR T cells to mediate MHC-unrestricted killing, as they are able to bind surface antigens. (4)

CAR T cell therapy has led to increased survival of cancer patients, in particular in patients with "liquid" hematologic malignancies (Table 1). Clinical trials have proven that CAR T cells are also suitable for various other cancer types. However, the results were underwhelming and showed limited efficacy for these solid tumors (Table 2).

The aim of this thesis is to determine why CAR T cell therapy works well for hematologic malignancies, but not for solid tumors. Possible reasons are the lack of suitable targets, inefficient trafficking or the immunosuppressive environment. Assessing the different obstacles will be done by looking at the efficacy, persistence and toxicity of CAR T-cells in hematologic and solid cancers. Novel strategies that might help overcome the obstacles in solid cancers will be highlighted

1 Design and development of CAR T cells

To understand why CAR T cell therapies work better for hematologic malignancies, it is essential to understand the designed and signaling mechanism of the CAR. The chimeric antigen receptor is a fusion protein that consists of an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain (Fig 1). The extracellular recognition domain is a single-chain variable fragment (scFv) that is derived from a monoclonal antibody.(5) The scFv domain allows the recognition of a specific tumor-associated antigen (TAA) by the T-cell. How well the T cell can recognize a TAA can be predicted by scFv affinity.(6) For instance, CAR T cells that contain a high affinity ROR1-specific scFv have an increased effector function (significantly higher IFN-γ TNF-α and IL-2 production) compared to low affinity scFvs(6). The intracellular signaling domain (often CD3-z) is the functional end of the receptor and stimulates T-cell proliferation, cytokine secretion and cytolysis upon TAA binding. (3)

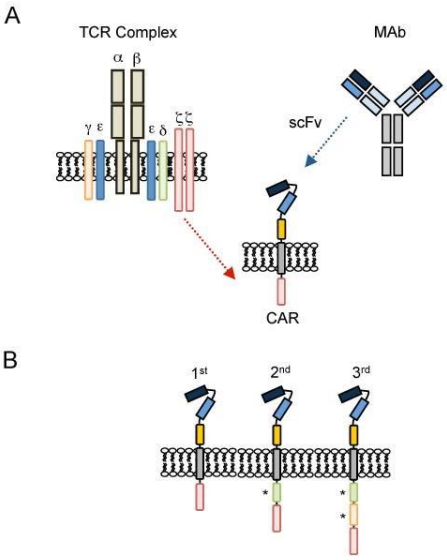

Figure 1: (A)The CARs consist of an ectodomain derived from a single chain variable fragment (scFv) a transmembrane domain and an endodomain (cd-3ζ). (B) CARs can be divided into different generations based on the number of costimulatory signaling domains. 1st generation (none), 2nd generation (one), and third generation (two).(6)

As shown in figure 1 B, CARs can be divided into three generations based on their structure of the endodomain (intracellular). The first generation of CARs used only a CD3ζ domain as the signaling endodomain.(7) The efficacy was low for CAR T cells in vivo because T-cells require not only interaction with their TCR, but also with other costimulatory receptors, such as CD28 and 4-1BB. The initial recognition of a specific antigen peptide triggers T cell receptor (TCR) signaling. However, it are the co-stimulatory and co-inhibitory receptors that control T cell function, and determine its fate. (3) This is stated in the classical two-signal hypothesis: both antigen recognition and secondary stimuli are required for full T cell activation.

The main problem of the first generation was a lack of co-stimulation.(1) This problem was improved in the second generation, by the addition of a costimulatory domain (4-1BB or CD28). Inclusion of 4-1BB in to the CAR architecture promotes the growth of CD8 central memory cells. These cells have significantly enhanced respiratory capacity, enhanced mitochondrial biogenesis and increased fatty acid oxidation. (3) The inclusion of the CD28 domain promotes the growth of effector memory cells with enhanced glycolysis. The addition of either CD28 or 4-1BB both significantly improved in vivo tumor killing, cytotoxicity, expansion and persistence in the second generation(1) Third generation contained two costimulatory domains. Similarly to the second generations the first domain is either CD28 or 4-1BB. The second domain was either CD28, 4-1BB or OXO40.Recently a fourth generation has been designed called the TRUCK CAR T cells. These CAR T cells are engineered to express cytokines that protect the CAR T cell from the immunosuppressive tumor microenvironment (8). This generation will be discussed further in the last chapter.

production

The production of CAR T cells is a process that requires multiple production steps. It is important that quality control testing is conducted during the entire production process. (9) The first step is to harvest leukocytes from the patients or donor's body by using leukapheresis. The T-cells are then enriched and separated from the other leukocytes. After activation with special beads they are ready for gene therapy. Gene therapy is needed to deliver the foreign CAR gene into the human T-cells. This can be achieved by either viral systems or non-viral systems. CARs are encoded with viral vectors, most commonly the lentiviral vectors (10). They integrate the foreign DNA into the T-cells by the use of reverse transcriptase. These T cells will then start to express the chimeric antigen receptors on their cell surface, and ultimately become CAR T-cells.(10)

2 CAR T cells for B cell/hematologic malignancies

CAR T cell therapy was first introduced by Eshhar et al., in 1993. There were initial attempts by other studies to treat patients with either solid or liquid tumors, but the real breakthrough of CAR T cells was achieved by targeting B-cell hematologic tumors(11). Thus far the most successful target of CAR T cell therapy for B-cell hematologic tumors has been CD19. Published studies have observed that CD19 targeted CAR T cells can reach a complete remission rate of 94% in both adults and children with refractory/relapsed acute lymphoblastic leukemia(ALL) (12). CD19 is a 95k transmembrane glycoprotein and is expressed in all stages of B cell development. (13) This means that CD19 is present on B-cell leukemias and lymphomas, healthy B-cells, but not hematopoietic stem cells or other tissues. This last characteristic makes CD19 an excellent anti-tumor target by minimizing off tumor toxicity. (13)

The use of second generation CD19 targeting CAR T cells have demonstrated high antitumor efficacy in patients with relapsed (R/R) B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL) and B-cell non-Hodgkin lymphoma (NHL). In Table 1 multiple studies involving CD19 targeted CAR T cells are shown.

Cancer type	Signaling Domain	No. of patients	Overall response rate	Complete remission (CR)	CRS incidence	Reference
B-ALL	CD3z-CD28	16 (Adults)	88%	63%	43% severe	14
B-ALL	CD3z-4-1BB	29 (Adults)	93%	86%	83% CRS 23% severe	15
DLBCL	CD3z-CD28	101	82%	58%	93%CRS 13% severe	16
B-ALL	CD3z-CD28	30 (Children and Adults)		90%	100% CRS 27% severe	17

Table 1: Outcomes of clinical trials with second generation CD19 targeted CAR T cells

As seen in table 1, overall response rates range from 82 to 93%, while complete remission is observed in 58-86%. Complete remission does not mean that these patients were cured. Maude et al., showed a 90% complete remission after the first month, but seven out of the 30 patients had a relapse between 6 and 36 weeks after infusion. The reasons for relapse are different per patient: Three were because of an early loss of detectable CD19 targeted CAR T cells. In three other patients the relapses were a result of the loss of CD19 expression in the leukemia cells. The 15 patients that did not have a relapse remained in remission, and received no further treatment. (17)

The success of the therapy led to the release two immunotherapies with anti-CD19 CAR T-cells by the FDA in 2007: KYMRIAH (tisagenlecleucel) and YESCARTA (axicabtagene ciloleacel). They are both second generation CAR T cells with different intracellular domains. They both have a CD3Zeta chain, but are fused with different costimulatory domains. Kymriah signals through a 4-1BB co-stimulatory domain, while Yescarta signals through a CD28 costimulatory domain.(1,11) KYMRIAH has been approved as a treatment for patients up to 25 years old with B-ALL, and YESCARTA is approved for adults with certain types of B-cell lymphoma. (1)

Side effects

Brudno et al., showed that the side effects or CAR-T cells are different from those of the other immune therapies. Checkpoint inhibitors have for example delayed and unpredictable side effects. CAR T cells on the other hand are less likely to have adverse effects, and have a more rapid onset.(18) However, there are still side effects from treatment with anti-CD19 CAR T cells. Two significant side effects are cytokine release syndrome (CRS) and neurotoxicity (NT), which have both been observed in patients treated with anti CD19 CAR T cells. (table 1)

CRS is an acute systemic inflammation that is caused by elevated inflammatory cytokines. Like every disease there are different degrees of CRS, each with corresponding cytokines. They range from mild CRS (constitutional symptoms) to severe CRS (sCRS: grade >3 organ toxicity, potentially life threatening). Symptoms include fevers, hypoxia and hypertension. CRS arises when large numbers of lymphocytes(B cells, T cells or NK cells) or myeloid cells (macrophages, monocytes etc.) become activated. Multiple studies observed highly increased cytokine levels after infusion of CAR T cells, including interferon-gamma, TNF-a, IL-10 and IL-6. (19,20,21) Even though all these cytokines contribute to the toxicity, evidence implicates that IL-6 is the central mediator of the toxicity. The current model holds that normal anti-inflammatory properties of IL-6 are mediated via classic signaling (activation of MAPK pathway), but that pro-inflammatory responses are a result of trans-signaling. Trans signaling occurs when IL-6 levels are elevated, which results in an activation of the soluble form of the IL-6 receptor. The soluble IL-6R can interact with a range of cells that normally have no response to IL-6. Therefore, trans signaling dramatically increases the spectrum of IL-6 target cells.(21)

Table 1 shows the incidence of CRS in studies that used CD19 targeted CAR cells. In reports/studies with CD19 targeted CAR T cells for relapsed/refractory B-ALL, the incidence sCRS has ranged from 19 to 43%. The variability is likely due to differences in CAR design, infused cell phenotype, or even clinical identification of the syndrome. Important to note is that the clinical outcome is not correlated with the development of sCRS. Patients may have an antitumor response without this toxicity. However, the majority of patients with hematologic malignancies that had an antitumor response exhibited at least mild CRS. (22) Neurological toxicity is the other toxicity that often occurs after CD19 specific CAR T cell infusion. Symptoms that have been reported include confusion, delirium, expressive aphasia and seizure. The pathophysiological mechanisms behind NT are currently unknown, but it is thought that the elevated levels of cytokines are partly responsible.(20) Other toxicities include anaphylaxis and on-target off tumor-toxicity (1).

3.1 CAR T cells for other types of cancer/solid tumors

As mentioned in the previous chapter, the CD19 directed CAR T cells have been very effective as a treatment for B-cell lymphoid malignancies (Table 1). This inspired many researchers to study the effects of CAR T cells on various other types of cancer. Unfortunately the use of CAR T cells for solid tumors has not been as successful. Multiple studies observed minimal clinical result of the CAR T cells(Table 2). For example, Kershaw et al., administered CAR T cells directed against the alpha-folate receptor (FR) receptor to fight metastatic ovarian cancer. They found that most patients (75%) tolerated the infusion well (mild side effects, grade 1 to 2 symptoms), but the anti-tumor effect was minimal due to lack of persistence. Most of the patients (91%) had little to no circulating CAR T cells at the end of the third week. As a result there was no reduction of tumor burden in any of the patients.(32)

Cancer subtype	**Target**	**No. of patients**	**Outcome**	**Persistence**		**Reference**
Neuroblastoma	GD2	19 (8 in remission, 11 active disease)	CR: 27% (3/11)	Up to 96 weeks		24
Metastatic ovarian cancer	FR	12	NR	3 weeks		23
Carcinomas	CD133	23 (progressive disease)	PR: 13% (3/23) SD: 61% (14/23) 21 out of 23 had developed no de novo lesions	Up to 2 months in 7 patients	Overall survival: 6 months (35%)	25
Sarcoma	HER2	17	SD:24% (4/17)	3 months in 4 patients 9 months in one patients	Median overall survival: 10.3 months	26

Table 2, Clinical trials of CAR T cells for the treatment of solid tumors.

The lack of clinical result of the CAR T cells for solid tumors is a multifactorial problem. The obstacles that need to be overcome are very different than those of the hematologic malignancies. There are four main obstacles/problems: First, solid tumors are very diverse. Every tumor expresses different specific tumor antigens in different amounts. Second, unlike in hematologic cancer, CAR T cells need to travel through the blood to the solid tumor site. Solid tumors can be hard to reach, and the CAR T cells can struggle to penetrate dense stroma. Third, CAR T cells need to overcome the constantly changing microenvironment of the solid tumor. Lastly, CAR T cells can develop T cell exhaustion, which can reduce the persistence of the CAR T cells.(1) The different obstacles will be discussed in the topics below. Additionally, promising studies and methods are highlighted that may be used to overcome these issues.

3.2 Tumor heterogeneity

The first challenge in developing CAR T cells for solid tumors is identifying a suitable target antigen. The ideal target antigen would be expressed on 100 % of the tumor cell surfaces, and not on any normal healthy tissue. However, solid tumors rarely express one tumor specific antigen. Each solid tumor, even if they are the same subtype, is genetically different. This is called tumor heterogeneity. Tumor heterogeneity can be divided into interpatient and intratumoral heterogeneity. Interpatient heterogeneity is a result of a combination of unique somatic or germ line mutations in a patient (average of 33 to 66 genes). For instance, Chen et al., performed direct sequencing to identify EGFR mutations in lung adenocarcinoma samples. In the study population (3071 patients) the discordance rate was 13.9% . Intratumoral heterogeneity means that there are distinct subpopulations within a tumor. These subpopulations have different genetic and epigenetic phenotypes. The differences become even bigger when comparing primary sites versus metastatic sites. The metastatic sites are highly genetically unstable, and often develop heterogeneity within the metastatic site (intra metastatic heterogeneity).(27) For instance, one metastatic lesion can have 20 clonal genetic alterations that are not shared by metastases in the same patient. (28) This means that response to CAR T cells therapy does not only vary significantly between patients, but also between tumor sites within the patient.

Even if there is currently a uniformly expressed TAA, there is still the possibility of antigen loss or antigen escape. This means that the antigen is no longer expressed on the tumor, which will cause relapses. Mejstrikova et al., showed that 22%(4/18) of their patients had CD19-negative relapses after CD19-CAR T treatment. Two mechanisms have been described to explain the antigen loss: loss of CD19 epitope or lineage switching. Loss of the CD19 epitope has occurred either through deletions within the CD19 gene, de novo frameshift and missense mutations in exon 2 of CD19, alternative splicing of CD19 mRNA. (6) Lineage switching occurs when B-precursor cells switch from a lymphoid lineage to a CD14-positive myeloid lineage, which occurs in 4% of B-precursor ALL.(29)

An additional problem that is not necessarily because of heterogeneity, but that is important to take in to consideration, is that the antigens expressed by solid tumors are often also found on normal healthy tissue. Examples of frequently targeted TAA's for solid tumors that are also found on healthy tissues include EGFR, CEA and ERBB2. (30) The lack of tumor antigen specificity means that the potential risk of on-target off-tumor toxicity is high for solid tumors. On-target off-tumor refers to the fact that normal tissue is attacked during treatment because of the shared expression of the target antigen. (31) Morgan et al., tried to treat a patient with metastatic colon cancer with an infusion of CAR T cells targeted to the above mentioned ERBB2 (HER2) antigen. The patient had respiratory problems after 15 minutes of injection, and died 5 days later after intensive medical intervention. According to the study the death was because of low levels of HER2 expression on the healthy epithelial cells of the patient's lungs, which were attacked by the CAR T cells.(32)

The study above shows that finding an appropriate and safe target antigen is essential, as low expression of target antigen on healthy tissue can cause major toxicity. It also showed that high affinity of the CAR T cells to the target antigen is not necessarily better. Park et al., confirmed this in their study when they used a ICAM-1 targeted CAR T cells. ICAM-1 is a biomarker that is associated with a variety of solid cancers, but is also expressed on normal tissue. The study showed that the CARs with a lower affinity (micromolar) were safer and more effective than those with a high affinity (nanomolar). (33)

Novel strategies

One strategy that is currently being explored to over overcome the lack of ideal TAA in solid tumors is the use of bispecific CARs. Thus Far three major classes of bispecific CARs have been used in T cell engineering: Dual CAR, tandem CAR (TanCAR) and inhibitory CAR (iCAR). These T cells express two or more CAR domains, that each have a different anti binding domain. This allows them to recognize multiple antigens at once. There are currently two strategies to this approach: the AND-strategy (Dual CARs), and the OR-strategy (Tandem CARs).(34) The AND-strategy requires the binding of two target antigens simultaneously, which means that Dual CARs will only be fully activated when both antigens are present on the tumor cell surface.(35) This is achieved by the use of synthetic Notch receptors. SynNotch receptors induce transcriptional activation in response to target antigen recognition. Roybal et al., used this concept to engineer a second CAR, that is under the control of a synNotch receptor. They used an anti-CD19 CAR T cells, and inserted the anti-mesothelin CAR gene. The synNotch system will induce transcription of this gene upon binding of the first antigen (CD19). This means that the secondary CAR, in this case anti-mesothelin, will only be expressed when the first antigen is present. The CAR T cell will be activated when the second antigen is also present. This was confirmed by the study, as they only observed upregulation of the marker CD69 and secretion of the cytokine IL-2 when the tumor cells expressed both CD19 and mesothelin (35). T cells can be targeted more specifically with this approach, because activation requires presence of multiple antigens on the tumor. This results in reduced off-target toxicity, as the CAR T cells will only activate on tissue that expresses the specific antigen combination.(34)

Hedge et al., initially to designed a similar AND-gate circuit that linked two distinct scFVs. However, their design ended up working as an OR-gate. They engineered CAR T cell that targeted both HER2 and IL13Ra2, by the use of a tandem CAR exodomain. They showed that these TanCAR T cells had comparable activation(20%lysis) to normal single CAR T cells when they encountered either HER2 or IL12a2. However, when the TanCARs bound to HER2 and IL13a2 simultaneously enhanced T cell activation was observed (increased cytotoxic ability, 60% lysis). (36) Furthermore, they also showed that these bispecific TanCARs mitigated antigen escape: They used a stress test experiment where large GBM xenografts were treated with circa 1 T cell per 30 tumor cells.Progression free survival (PFS) was used as primary outcome,and overall survival (OS) as secondary. Treatment with HER2 CAR T cells and IL13Ra2 CAR T cells showed a median PFS of 14 days. Treatment with TanCAR T cells extended the PFS to 36 days. The median OS after treatment with HER2 CAR T cells and IL13Ra2 CAR T cells was 54 days, while treatment with TanCAR T cells extended the OS to 86 days. They also assed antigen expression after treatment with staining and showed that unispecific treatment downregulated or even eliminated target expression, while treatment with TanCAR showed dim staining of both HER2 andI L13Ra2.(36) This confirms that TanCARs do not only enhance CAR T cell activation, but also mitigate antigen loss, making them an promising strategy for future research.

3.3 Inefficient trafficking of T cells to tumor site

Once a CAR T cell with an appropriate target antigen has been generated and infused into the patient, a second obstacle occurs: the CAR T cells need to be able to migrate and infiltrate the solid tumor. It is suggested that poor migration and infiltration of the CAR T cells are one of the reasons of the low clinical response for solid tumors (25, 37)Unlike in hematologic cancer, CAR T cells need to travel through the blood to the tumor site. Once they have accumulated in the area surrounding the tumor, they still need to infiltrate the tumor. This means that the CARs must cross various barriers in order to reach the tumor. This can create problems as certain barriers are very hard to cross, e.g the stroma (high tissue pressure that). (38)

The ability of CAR T cells to migrate successfully to the tumor site is dependent on appropriate expression of adhesion receptors on both the CAR T cell and the tumor endothelium. It also requires a match between the chemokines secreted by the tumor, and the chemokine receptors on the CAR T cells (often CXCR3 and CCR5) (37). Unfortunately there is often a mismatch between these chemokines and chemokine receptors, which leads to decreased migration. (38)For instance, CAR T cells that express a high amount of CXCR3 will have inefficient targeting of a tumor that produces small amounts of CXCR3 ligands.

Novel strategies
Various efforts have been made to enhance CAR-T cell migration to tumor sites. For instance, there have been attempts to create a match between the chemokines that are produced by the tumor and the chemokine receptors on the T-cells. Matching the unique variation of chemokines secreted by the tumor, with appropriate receptors on the CARs might enhance T-cell migration. (39) Kershaw et al., investigated a strategy to redirect T cells toward chemokines expressed by the tumor. They used Gro-Alpha as a target chemokine because it was produced by the tumor, but not by the T cell. The used T cells were engineered to express the receptor for Gro-Alpha, CXCR2, as they did not express it naturally. The researchers found that the addition of the CXCR2 receptor showed increased migration (65%) toward the tumor site. (40)

Craddock et al., did a similar study and investigated if the tumor trafficking for a GD2 targeted CAR T cell could be enhanced by the addition of the chemokine receptor CCR2b. The CCR2b receptor directs migration towards the chemokine CCL2, which is produced by various tumors. In the study neuroblastoma cells were used that secreted a high amount of CCL2. They indicated that GD2-CARs had low expression of the CCR2 (<5%) receptor, and thus poor migration to the neuroblastoma cells. However, after retroviral transduction the CAR T cells had high CCR2b receptor expression (>60%), and the CARs migrated well to the tumor cells in vitro. They also tested in vivo and observed that CCR2b positive GD2-CARs had improved homing (>10-fold) towards CCL2-secreting neuroblastoma compared to CCR2b negative GD2-CARs. (41)

The addition of chemokine receptors is not the only way of improving T cell trafficking. A different option that is being explored is the local instillation of CARs. Adusumilli et al., compared the systemic to the regional delivery of mesothelin-targeted CAR T cells . The intra-pleural administered CAR T cells showed enhanced antitumor efficacy and increased persistence (38) Intravenous injected CAR T cells did not achieve comparable tumor eradication or persistence, even when the CAR T cells accumulated at equivalent numbers in the tumor. Additionally, they found that intra-pleural delivered CAR T cells required 30-fold less CAR T cells to induce long term complete remissions. (38) Even though this study shows multiple advantages of local injection, there are still disadvantages compared to intravenous administration. For instance, local instillation is often more technically challenging than intravenous injection. Furthermore, site-specific injection of will likely result in higher CAR T cells locally, but these CARs will face the same issues as intravenous injected CAR T cells when they need to migrate to other tumor sites (38).

3.4 Immunosuppressive microenvironment

Even if the CAR T cells are able to migrate to the tumor site, they still have to overcome the immunosuppressive tumor microenvironment (TME). Tumors consist of malignant cancer cells, stromal cells like fibroblasts, and immune cells. Together they make up the TME, which is essential for tumor growth and spread by preventing immune-mediated destruction. (42) There are multiple mechanisms and factors involved in this process that are all targets for next generation CART therapeutics that escape these processes.

One of the mechanisms involves the dysregulated growth of the cancer cells. The TME is characterised by nutrient depletion, regions of hypoxia and acidic pH. (1)The acidic pH is a result of elevated levels of lactate, which are due to enhanced glycolysis in the cancer cells. (1) Fischher et al., examined the influence of lactic acid produced by the tumor on the immune functions in vitro. They found that the lactic acid suppressed the cytokine production of human CTLs up to 95%. This resulted in a 50% decrease in cytotoxic activity. They concluded that the high lactic acid concentration in the tumors blocked the lactic acid export in the T-cells, which disturbed their metabolism and function. (43)

Furthermore, nutrient starvation in the area of the solid tumor can lead to low levels of essential amino acids and low glucose levels. Tryptophan is one of the amino acids that is essential for proper T-cell function, but is decreased in the TME region. This is because tryptophan is catabolized into kynurenine by an enzyme called IDO (indoleamine 2,3-dioxygenase). The low tryptophan levels lead to mTOR inhibition which in turn makes the T-cells inactive. IDO has been observed in several cancer types and is produced by both the tumor and other immunoregulatory cells.(45) Ninomiya et al., showed that CD19 CAR T-cells can fail to control IDO-expressing lymphoma, so antagonizing the IDO enzyme might benefit CD19-CAR T cell therapy, and other CAR T-cell therapies in the future. (44)

Immunoregulatory cells also contribute to the hostile and immunosuppressive TME. Various immunoregulatory cells are present in the TME including: regulatory T cells (Tregs), Tumor associated neutrophils (TANS) and Tumor associated macrophages (TAMS). TANS and TAMS in combination with Tregs produce immunosuppressive cytokines/ligands like TGFB, PD-L1, reactive oxygen species and arginase. (46). All of these factors have the ability to decrease T cell mediated tumor immunity, and thus enhance tumor escape. For instance, TGF-B (transforming growth factor B) can function either as tumor promoter, or suppressor. As a tumor promoter it enhances tumor proliferation by suppressing the antitumor response of CD8 CTLs in the TME. Huang et al., showed this by measuring TGF-B and CD8 CTLs levels with immunohistochemical staining, and comparing it with the overall survival. They observed no correlation between TGF-B levels and CD8 CTLs density, but did show that the survival of patients was only improved when TGF-B levels were low. (47) All of the above mechanisms make CAR T cell treatment for solid tumors challenging, as the TME impedes the CAR T proliferation and their ability to engage with their target antigen (1)

Novel strategies

One promising strategy that is being explored aims to give CAR T cells the ability to remodel the tumor suppressive environment by secreting anti-cancer cytokines. These are the fourth generation CAR T cells named TRUCK (T cells redirected for universal cytokine-mediated killing). Cytokines that have been adopted into TRUCK systems include IL-12, IL18 and TNFRSF14. (34). Chmielowski et al., engineered cytotoxic T cells to release IL-12 upon CAR engagement in the tumor region. This resulted in recruitment of activated macrophages, enhanced inflammatory response.(48) Additionally, the IL-12 release also resulted in the destruction of antigen loss cancer cells that would not have been recognized by the CAR T cells. Koneru et al., had a similar approach and showed that IL-12 secreting MUC-16 targeted CAR T cells had enhanced antitumor efficacy (increased survival, prolonged persistence)

Roybal et al., developed a leading edge strategy by combining TRUCK with the earlier discussed synNotch receptors. These TRUCKs were able to produce specific therapeutic payloads in response to target antigens. The flexible synNotch system allowed them to express a range of different therapeutics, including inflammatory cytokines, checkpoint antibodies and bispecific antibodies(8) So the synNotch receptor does not only increase T cell specificity (topic 3.2), it can also be used to remodel local microenvironments associated with various diseases (8).

3.5 CAR T cell persistence

The last obstacle that needs to be overcome is the lack of CAR T cell persistence in solid tumors. Research has shown that CAR T cells can persist for multiple months, or even years in patients with hematologic malignancies (Table 1). Unfortunately this is not the case for solid tumors. Clinical trials showed that patients had little to no detectable CAR T cells only a couple months or even weeks after administration (Table 2). Feng et al., did a clinical study with EGFR-targeted CAR T cells in patients with non-small lung cancer. The 11 available patients received a median dose of 0.97x10(7) CAR T cells by transfusion. In 4 of the 11 patients the EGFR-CAR T cells were detectable for only 11 to 34 days. In contrast, the other 7 patients had detectable CAR T cells for 7 to 37 weeks. (49)
In a similar study Wang et al., looked at the effect of CD133 targeted CARs on advanced metastatic malignancies. They observed that after two months low level signals could be detected in only 7 out of the 23 patients. These two studies, and the studies in table 2 show that CAR T cells have poor persistence in solid tumors.

Conclusion

CD19 targeted CAR T cell therapy has shown to be an effective treatment for hematological cancers, especially for relapsed/refractory B-ALL. The success of the clinical studies led to the approval of the first two immunotherapies with anti CD19-CAR T cells by the FDA. Novel clinical trials have shown that CAR T cells are safe and feasible for patients with solid tumors, but have shown limited results. This is because CAR T cells need to overcome additional obstacles in solid tumors. The obstacles that have been discussed are the following. The first obstacle is the heterogeneity of solid tumors. Solid tumors rarely express a unique antigen, which is due to interpatient and intratumoral heterogeneity. Additionally, solid tumors often express antigens that are also expressed on healthy tissue, which makes finding a specific antigen without increase on-target off-tumor toxicity more difficult. Bispecific dual and tandem CAR T cells have shown to be a promising way of increasing tumor antigen recognition specificity, an thus decreasing on-target off-tumor toxicity. They also showed mitigated antigen loss, making these designs a promising strategy for future treatments. The second obstacle is the poor migration of CAR T cells to the tumor site. A match between adhesins, chemokines and chemokine receptors is needed for effective trafficking, which is often lacking in solid tumors. The novel strategy of implementing a additional chemokine receptor in the CAR T cell has shown increased migration towards the tumor. Future exploration of this strategy might reveal promising chemokine receptors that can be used to increase migration further. The third obstacle is the immunosuppressive tumor environment. The TME makes CAR T cell treatment for solid tumors challenging, as the it impedes the CAR T proliferation and their ability to engage with their target antigen. Additional modification to the CAR T cells might be necessary to overcome the immunosuppressive microenvironment. Strategies that implement the secretion of cytokines or other therapeutics under control of synNotch receptors have shown promising results for survival in the TME. Lastly, the fourth obstacle is limited CAR T cell persistence in solid tumors. Thus far novel clinical studies have shown promising results and strategies to improve the treatment of solid tumors with CAR T cells. However, the CAR designs need to be further optimized in order to have similar tumor control as hematologic cancers. Improvements in CAR T cell designs and better understanding of tumor-immune system interactions are needed to overcome to obstacles that currently limit CAR T cell treatment in solid tumors.

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References

1. Heyman, B., & Yang, Y. (2019). Chimeric Antigen Receptor T Cell Therapy for Solid Tumors: Current Status, Obstacles and Future Strategies. Cancers, 11(2), 191. https://doi.org/10.3390/cancers11020191

2. Novel immunotherapies in lymphoid malignancies. Batlevi CL, Matsuki E, Brentjens RJ, Younes Nat Rev Clin Oncol. 2016 Jan; 13(1):25-40

3.Kawalekar, O. U., O'Connor, R. S., Fraietta, J. A., Guo, L., McGettigan, S. E., Posey, A. D., Jr., … June, C. H. (2016). Distinct Signaling of Coreceptors Regulates Specific Metabolism Pathways and Impacts Memory Development in CAR T Cells. Immunity, 44(2), 380–390. https://doi.org/10.1016/j.immuni.2016.01.021

4.Benmebarek, M.-R., Karches, C., Cadilha, B., Lesch, S., Endres, S., & Kobold, S. (2019). Killing Mechanisms of Chimeric Antigen Receptor (CAR) T Cells. International Journal of Molecular Sciences, 20(6), 1283. https://doi.org/10.3390/ijms20061283

5. Brudno, J. N., & Kochenderfer, J. N. (2017). Chimeric antigen receptor T-cell therapies for lymphoma. Nature Reviews Clinical Oncology, 15(1), 31–46. https://doi.org/10.1038/nrclinonc.2017.128

6.Mejstríková, E., Hrusak, O., Borowitz, M. J., Whitlock, J. A., Brethon, B., Trippett, T. M., … Locatelli, F. (2017). CD19-negative relapse of pediatric B-cell precursor acute lymphoblastic leukemia following blinatumomab treatment. Blood Cancer Journal, 7(12), 1. https://doi.org/10.1038/s41408-017-0023-x

7.Chimeric Fv-zeta or Fv-epsilon receptors are not sufficient to induce activation or cytokine production in peripheral T cells, Brocker T., Blood. 2000 Sep 1; 96(5):1999-2001.

8.Roybal, K. T., Williams, J. Z., Morsut, L., Rupp, L. J., Kolinko, I., Choe, J. H., … Lim, W. A. (2016). Engineering T Cells with Customized Therapeutic Response Programs Using Synthetic Notch Receptors. Cell, 167(2), 419-432.e16. https://doi.org/10.1016/j.cell.2016.09.011

9. Levine, B. L. (2015). Performance-enhancing drugs: design and production of redirected chimeric antigen receptor (CAR) T cells. Cancer Gene Therapy, 22(2), 79–84. https://doi.org/10.1038/cgt.2015.5

10.McGarrity GJ, Hoyah G, Winemiller A, Andre K, Stein D, Blick G, et al. Patient monitoring and follow-up in lentiviral clinical trials. J Gene Med. 2013;15(2):78–82.

11. D'Aloia, M. M., Zizzari, I. G., Sacchetti, B., Pierelli, L., & Alimandi, M. (2018). CAR-T cells: the long and winding road to solid tumors. Cell Death & Disease, 9(3). https://doi.org/10.1038/s41419-018-0278-6

12. Wei, G., Ding, L., Wang, J., Hu, Y., & Huang, H. (2017). Advances of CD19-directed chimeric antigen receptor-modified T cells in refractory/relapsed acute lymphoblastic leukemia. Experimental Hematology & Oncology, 6(1), 1. https://doi.org/10.1186/s40164-017-0070-9

13 .Wang, K., Wei, G., & Liu, D. (2012). CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. Experimental Hematology & Oncology, 1(1), 36. https://doi.org/10.1186/2162-3619-1-36

14. Davila, M. L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K., … Brentjens, R. (2014b). Efficacy and Toxicity Management of 19-28z CAR T Cell Therapy in B Cell Acute Lymphoblastic Leukemia. Science Translational Medicine, 6(224), 224ra25. https://doi.org/10.1126/scitranslmed.3008226

15 Turtle, C. J., Hanafi, L.-A., Berger, C., Gooley, T. A., Cherian, S., Hudecek, M., … Maloney, D. G. (2016). CD19 CAR–T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. Journal of Clinical Investigation, 126(6), 2123–2138. https://doi.org/10.1172/jci85309

16 Neelapu, S. S., Locke, F. L., Bartlett, N. L., Lekakis, L. J., Miklos, D. B., Jacobson, C. A., … Go, W. Y. (2017). Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. New England Journal of Medicine, 377(26), 2531–2544. https://doi.org/10.1056/nejmoa1707447

17 Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., … Grupp, S. A. (2014). Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia. New England Journal of Medicine, 371(16), 1507–1517. https://doi.org/10.1056/nejmoa1407222

18.Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016:blood-2016-04-703751

19. Davila, M. L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K., … Brentjens, R. (2014). Efficacy and Toxicity Management of 19-28z CAR T Cell Therapy in B Cell Acute Lymphoblastic Leukemia. Science Translational Medicine, 6(224), 224ra25. https://doi.org/10.1126/scitranslmed.3008226

20. Bonifant, C. L., Jackson, H. J., Brentjens, R. J., & Curran, K. J. (2016). Toxicity and management in CAR T-cell therapy. Molecular Therapy - Oncolytics, 3, 16011. https://doi.org/10.1038/mto.2016.11

21. Lee, D. W., Gardner, R., Porter, D. L., Louis, C. U., Ahmed, N., Jensen, M., … Mackall, C. L. (2014). Current concepts in the diagnosis and management of cytokine release syndrome. Blood, 124(2), 188–195. https://doi.org/10.1182/blood-2014-05-552729

22. Lee, D. W., Kochenderfer, J. N., Stetler-Stevenson, M., Cui, Y. K., Delbrook, C., Feldman, S. A., … Mackall, C. L. (2015). T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. The Lancet, 385(9967), 517–528. https://doi.org/10.1016/s0140-6736(14)61403-3

23. Kershaw, M. H., Westwood, J. A., Parker, L. L., Wang, G., Eshhar, Z., Mavroukakis, S. A., … Hwu, P. (2006). A Phase I Study on Adoptive Immunotherapy Using Gene-Modified T Cells for Ovarian Cancer. Clinical Cancer Research, 12(20), 6106–6115. https://doi.org/10.1158/1078-0432.ccr-06-1183

24. Louis, C. U., Savoldo, B., Dotti, G., Pule, M., Yvon, E., Myers, G. D., … Brenner, M. K. (2011). Antitumor activity and long-term fate of chimeric antigen receptor–positive T cells in patients with neuroblastoma. Blood, 118(23), 6050–6056. https://doi.org/10.1182/blood-2011-05-354449

25. Wang, Y., Chen, M., Wu, Z., Tong, C., Dai, H., Guo, Y., … Han, W. (2018b). CD133-directed CAR T cells for advanced metastasis malignancies: A phase I trial. OncoImmunology, 7(7), e1440169. https://doi.org/10.1080/2162402x.2018.1440169

26. Ahmed, N., Brawley, V. S., Hegde, M., Robertson, C., Ghazi, A., Gerken, C., … Gottschalk, S. (2015). Human Epidermal Growth Factor Receptor 2 (HER2) –Specific Chimeric Antigen Receptor–Modified T Cells for the Immunotherapy of HER2-Positive Sarcoma. Journal of Clinical Oncology, 33(15), 1688–1696. https://doi.org/10.1200/jco.2014.58.0225

27.Gerlinger, M., Rowan, A. J., Horswell, S., Larkin, J., Endesfelder, D., Gronroos, E., … Swanton, C. (2012). Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. New England Journal of Medicine, 366(10), 883–892. https://doi.org/10.1056/nejmoa111320

28. Vogelstein, B., Papadopoulos, N., Velculescu, V. E., Zhou, S., Diaz, L. A., & Kinzler, K. W. (2013). Cancer Genome Landscapes. Science, 339(6127), 1546–1558. https://doi.org/10.1126/science.1235122

29.Slamova, L., Starkova, J., Fronkova, E., Zaliova, M., Reznickova, L., van Delft, F. W., … Mejstrikova, E. (2013). CD2-positive B-cell precursor acute lymphoblastic leukemia with an early switch to the monocytic lineage. Leukemia, 28(3), 609–620. https://doi.org/10.1038/leu.2013.354

30. Martinez, M., & Moon, E. K. (2019). CAR T Cells for Solid Tumors: New Strategies for Finding, Infiltrating, and Surviving in the Tumor Microenvironment. Frontiers in Immunology, 10, 1. https://doi.org/10.3389/fimmu.2019.00128

31.Sun, S., Hao, H., Yang, G., Zhang, Y., & Fu, Y. (2018). Immunotherapy with CAR-Modified T Cells: Toxicities and Overcoming Strategies. Journal of Immunology Research, 2018, 1–10. https://doi.org/10.1155/2018/2386187

32.Morgan, R. A., Yang, J. C., Kitano, M., Dudley, M. E., Laurencot, C. M., & Rosenberg, S. A. (2010). Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing ERBB2. Molecular Therapy, 18(4), 843–851. https://doi.org/10.1038/mt.2010.24

33. Park, S., Shevlin, E., Vedvyas, Y., Zaman, M., Park, S., Hsu, Y.-M. S., … Jin, M. M. (2017). Micromolar affinity CAR T cells to ICAM-1 achieves rapid tumor elimination while avoiding systemic toxicity. Scientific Reports, 7(1), 1. https://doi.org/10.1038/s41598-017-14749-3

34.Hartmann, J., Schüßler-Lenz, M., Bondanza, A., & Buchholz, C. J. (2017). Clinical development of CAR T cells—challenges and opportunities in translating innovative treatment concepts. EMBO Molecular Medicine, 9(9), 1183–1197. https://doi.org/10.15252/emmm.201607485

35.Roybal, K. T., Rupp, L. J., Morsut, L., Walker, W. J., McNally, K. A., Park, J. S., & Lim, W. A. (2016). Precision Tumor Recognition by T Cells With Combinatorial Antigen-Sensing Circuits. Cell, 164(4), 770–779. https://doi.org/10.1016/j.cell.2016.01.011

36. Hegde, M., Mukherjee, M., Grada, Z., Pignata, A., Landi, D., Navai, S. A., … Ahmed, N. (2016). Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate tumor antigen escape. Journal of Clinical Investigation, 126(8), 3036–3052. https://doi.org/10.1172/jci83416

37. Harlin, H., Meng, Y., Peterson, A. C., Zha, Y., Tretiakova, M., Slingluff, C., … Gajewski, T. F. (2009). Chemokine Expression in Melanoma Metastases Associated with CD8+ T-Cell Recruitment. Cancer Research, 69(7), 3077–3085. https://doi.org/10.1158/0008-5472.can-08-2281

38. Adusumilli, P. S., Cherkassky, L., Villena-Vargas, J., Colovos, C., Servais, E., Plotkin, J., … Sadelain, M. (2014). Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. Science Translational Medicine, 6(261), 261ra151. https://doi.org/10.1126/scitranslmed.3010162

39.Zhang, B.-L., Qin, D.-Y., Mo, Z.-M., Li, Y., Wei, W., Wang, Y.-S., … Wei, Y.-Q. (2016). Hurdles of CAR-T cell-based cancer immunotherapy directed against solid tumors. Science China Life Sciences, 59(4), 340–348. https://doi.org/10.1007/s11427-016-5027-4

40.Kershaw, M. H., Wang, G., Westwood, J. A., Pachynski, R. K., Tiffany, H. L., Marincola, F. M., … Hwu, P. (2002). Redirecting Migration of T Cells to Chemokine Secreted from Tumors by Genetic Modification with CXCR2. Human Gene Therapy, 13(16), 1971–1980. https://doi.org/10.1089/10430340260355374

41.Craddock, J. A., Lu, A., Bear, A., Pule, M., Brenner, M. K., Rooney, C. M., & Foster, A. E. (2010). Enhanced Tumor Trafficking of GD2 Chimeric Antigen Receptor T Cells by Expression of the Chemokine Receptor CCR2b. Journal of Immunotherapy, 33(8), 780–788. https://doi.org/10.1097/cji.0b013e3181ee6675

42. Tormoen, G. W., Crittenden, M. R., & Gough, M. J. (2018). Role of the immunosuppressive microenvironment in immunotherapy. Advances in Radiation Oncology, 3(4), 520–526. https://doi.org/10.1016/j.adro.2018.08.018 11.https://www.ncbi.nlm.nih.gov/pubmed/17255361/

43 Fischer, K., Hoffmann, P., Voelkl, S., Meidenbauer, N., Ammer, J., Edinger, M., … Kreutz, M. (2007). Inhibitory effect of tumor cell–derived lactic acid on human T cells. *Blood*, *109*(9), 3812–3819. https://doi.org/10.1182/blood-2006-07-035972

44 Ninomiya, S., Narala, N., Huye, L., Yagyu, S., Savoldo, B., Dotti, G., … Ramos, C. A. (2015). Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. Blood, 125(25), 3905–3916. https://doi.org/10.1182/blood-2015-01-621474

45 Brochez, L., Chevolet, I., & Kruse, V. (2017). The rationale of indoleamine 2,3-dioxygenase inhibition for cancer therapy. European Journal of Cancer, 76, 167–182. https://doi.org/10.1016/j.ejca.2017.01.011

46. Newick, K., Moon, E., & Albelda, S. M. (2016). Chimeric antigen receptor T-cell therapy for solid tumors. Molecular Therapy - Oncolytics, 3, 16006. https://doi.org/10.1038/mto.2016.6

47. Huang, C., Wang, H., Liao, W., Han, F., Li, Y., Chen, S., & Lao, X. (2017). Transforming Growth Factor β is a Poor Prognostic Factor and Inhibits the Favorable Prognostic Value of CD8+ CTL in Human Hepatocellular Carcinoma. *Journal of Immunotherapy*, *40*(5), 175–186. https://doi.org/10.1097/cji.0000000000000166

48.Chmielewski, M., Kopecky, C., Hombach, A. A., & Abken, H. (2011). IL-12 Release by Engineered T Cells Expressing Chimeric Antigen Receptors Can Effectively Muster an Antigen-Independent Macrophage Response on Tumor Cells That Have Shut Down Tumor Antigen Expression. Cancer Research, 71(17), 5697–5706. https://doi.org/10.1158/0008-5472.can-11-0103

49 Wang, Y., Chen, M., Wu, Z., Tong, C., Dai, H., Guo, Y., … Han, W. (2018). CD133-directed CAR T cells for advanced metastasis malignancies: A phase I trial. OncoImmunology, 7(7), e1440169. https://doi.org/10.1080/2162402x.2018.1440169