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Therapeutic advantage of combinatorial CAR T cell and chemo-therapies

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Non-standard abbreviations

ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
B-ALL	B cell acute lymphocytic leukemia
CAF	cancer associated fibroblast
CAR	Chimeric Antigen Receptor
CLL	chronic lymphocytic leukemia
CML	chronic myeloid leukemia
CRS	cytokine release syndrome
DIPG	diffuse intrinsic pontine glioma
ECM	extra cellular matrix
ESCC	esophageal squamous cell carcinoma
FDA	Food and Drug Administration
GBM	glioblastoma
HSC	hematopoietic stem cell
ICANS	immune effector cell-associated neurotoxicity syndrome
ITAMs	immunoreceptor tyrosine-based activation motifs
MCL	mantle cell lymphoma
MDSCs	myeloid derived suppressor cells
MM	multiple myeloma
NB	neuroblastoma
NK	natural killer
NSCLC	non-small cell lung carcinoma
SEAKER	synthetic enzyme armed killer
TAM	tumor associated macrophages
TCR	T Cell Receptor
TNBC	triple negative breast cancer
Treg	T regulatory cell

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Abstract:

Chimeric antigen receptor (CAR) T cell therapies have transformed outcomes for many patients with hematological malignancies. However, some patients do not respond to CAR T cell treatment, and adapting CAR T cells for solid and brain tumors has been met with many challenges including a hostile tumor microenvironment and poor CAR T cell persistence. Thus, it is unlikely that CAR T cell therapy alone will be sufficient for consistent, complete tumor clearance across cancer patients. Combinatorial therapies of CAR T cells and chemotherapeutics are a promising approach for overcoming this as chemotherapeutics could augment CAR T cells for improved anti-tumor activity or work in tandem with CAR T cells to clear tumors. Herein, we review efforts towards achieving successful CAR T cell and chemical drug combination therapies. We focus on combination therapies with approved chemotherapeutics as these will be more easily translated to the clinic, but also review non-approved chemotherapeutics and drug screens designed to reveal promising new CAR T cell and chemical drug combinations. Together, this review highlights the promise of CAR T cell and chemotherapy combinations with specific focus on how combinatorial therapy overcomes challenges faced by either monotherapy and supports the potential of this therapeutic strategy to improve outcomes for cancer patients.

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Significance Statement: Improving currently available CAR T cell products via combinatorial therapy with chemotherapeutics has the potential to drastically expand the types of cancers and number of patients that could benefit from these therapies when neither alone has been sufficient to achieve tumor clearance. Herein, we provide a thorough review of the current efforts towards studying CAR T and chemotherapy combinatorial therapies and provide perspectives on optimal ways to identify new and effective combinations moving forward.

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I. Introduction

Despite major advances in cancer research and treatment in just over 50 years since the signing of the National Cancer Act in 1971, cancer remains a significant health care crisis in the United States and worldwide. The American Cancer Society predicts that in 2024, over 2 million Americans will be diagnosed with cancer, a record-breaking number. While cancer death rates are declining slightly, still over 600,000 cancer related deaths are predicted this year (Siegel et al., 2024). Moreover, the decline in death rate is highly dependent on the cancer diagnosis. For example, recent years have made significant progress in curing patients with lung and stomach cancer; but prostate and central nervous system cancer death rates have not significantly improved (Siegel et al., 2023). Continued advancement in cancer treatment is needed to meet this growing health challenge.

As molecular tools for understanding oncogenesis across cancer types have developed, targeted chemotherapeutics have been designed to tailor a treatment plan towards a specific diagnosis (Anand et al., 2023). However, some cancers have yet to see an effective targeted chemotherapy developed or chemotherapeutics do not elicit curative responses on their own. Some of the major challenges faced by chemotherapeutics are lack of tissue specificity, limited half-life in the body, primary or acquired resistance, and significant short term and long term off-tumor toxicities (Anand et al., 2023; Chakraborty and Rahman, 2012). For example, primary or metastatic brain tumors are difficult to target due to the lack of penetrance of chemotherapeutics across the blood-brain-barrier (Terceiro et al., 2023; Upton et al., 2022). This severely limits the local concentration at the tumor site and renders chemotherapeutics ineffective. Chemotherapeutics often have short half-lives in the body (Lorscheider et al., 2021). Systemic delivery means that the drugs must be stable long enough reach the site of the tumor and retain activity. However, this produces an additional challenge – many healthy organs, tissues, and circulating cells are exposed to the active chemotherapeutic, leading to the myriad adverse side effects many patients experience (Nurgali et al., 2018). Ideally, anti-cancer treatments will exhibit anti-tumor potency and be tissue penetrant, biologically stable, and minimize off-target effects.

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In recent years, the number of annual FDA approvals of new cytotoxic chemotherapeutics has largely flatlined, while the approval rate of targeted biologics and immunotherapies skyrocketed (Scott et al., 2023). Targeted biologics and immunotherapies have been shown to safely and specifically target tumor cells, limiting off-tumor toxicities and rendering them more tolerable than traditional chemotherapeutics (Schirrmacher, 2019; Waldman et al., 2020). The first immune-based therapy for cancer to gain FDA approval was the administration of interferon-alpha 2 (IFN-α2) to stimulate an anti-cancer response through both the innate and adaptive immune systems (Brassard et al., 2002; Eno, 2017). Since then, various monoclonal antibodies, vaccines, and cell-based therapies have been approved as anti-cancer treatments (Twomey and Zhang, 2021). One mechanism for these therapies is to either prime (vaccines) or re-invigorate/boost (monoclonal antibodies) the endogenous immune system to fight a tumor (Hargrave et al., 2023). Another is to isolate anti-tumorigenic immune effector cells from a patient, expand them *ex vivo,* and reinfuse them into the patient (Morotti et al., 2021). This strategy relies on the identification of an endogenous effector cell (T or natural killer) that has successfully infiltrated the tumor and has anti-tumor activity (Brummel et al., 2023; Laskowski et al., 2022). To broaden the applicability of the approach, researchers developed chimeric antigen receptors (CARs), synthetic molecules that recognize tumor specific antigens and are transduced into peripheral-isolated effector cells to elicit a targeted cytotoxic response (Albinger et al., 2021; Eshhar et al., 1993; Feins et al., 2019). This strategy does not rely on isolating endogenous tumor-reactive immune effector cells and is highly modular. Engineered T cell therapy is currently gaining the most traction with FDA approvals and CAR T cell therapy is one of the primary focuses of this review.

II. CAR T Cell Therapy

CAR T cells are isolated T cells that are genetically engineered to express CARs. Briefly, the CAR molecule is loosely based on the natural cytotoxic T cell receptor (TCR) and recognizes tumor specific antigens on the surface of tumor cells and transduces the recognition signal into activation of T cell cytotoxicity (**Fig.1A**). CAR targets are identified based on high tumor specific expression and low

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healthy tissue expression, limiting the potential of on-target off-tumor toxicity (Wang and Rivière, 2016; Yee, 2014). CAR T cell therapy has displayed promising success in treating certain hematologic malignancies, having received 6 approvals by the FDA (Kymriah™, Yescarta™, Tecartus™, Breyanzi™, Abecma™, and Carvykti™), and is actively being investigated as treatment for additional blood borne cancers, solid tumors, and brain tumors (Asmamaw Dejenie et al., 2022; Grupp et al., 2013; Kochenderfer et al., 2015; Porter et al., 2011).

Manufacturing CAR T cells begins by harvesting peripheral blood mononuclear cells from patients (clinically) or healthy donors (pre-clinically). T cells are isolated and activated, virally transduced to express the CAR of interest, and expanded for infusion or pre-clinical testing (Wang and Rivière, 2016). Structurally, CARs have four primary domains: the antigen recognition domain, hinge region, transmembrane domain, and signaling domain (Jayaraman et al., 2020; Sadelain et al., 2013; Zhang et al., 2017a). The antigen recognition domain (**Fig.1B**) orients extracellularly and is responsible for CAR T cell recognition of the target antigen. Most CAR T cell constructs incorporate the single chain variable fragment (scFv) of a highly specific monoclonal antibody for antigen recognition. Newer CAR designs have also utilized endogenous binding partners or peptide sequences known to strongly bind a target (Asmamaw Dejenie et al., 2022; Hebbar et al., 2022; Ibanez et al., 2023).

The hinge region (**Fig.1C**) is a spacer between the antigen recognition and transmembrane domains. It facilitates antigen binding and signal transduction by imparting flexibility and adding length, making surface antigens more accessible and reducing rigidity and steric hinderance (Guest et al., 2005). Ultimately, the hinge domain has been found to regulate the threshold at which antigen can be recognized and downstream signaling is initiated (Fujiwara et al., 2020).

The transmembrane domain (**Fig.1D**) is a crucial mediator for relaying extracellular antigen binding to intracellular signal transduction. It anchors the construct and offers essential stability for the CAR T cell. Selection of the transmembrane domain determines the overall surface expression level of

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the CAR construct as well as recruitment of other signal-propagating components found in the native TCR (Fujiwara et al., 2020).

The signaling domain orients intracellularly and imparts the effector function of the CAR T cell upon antigen binding. The intracellular domain commonly consists of both co-stimulatory support (**Fig.1E**) and cytotoxic signaling components (**Fig.1F**) (Zhang et al., 2017a). Phosphorylation of the three immunoreceptor tyrosine-based activation motifs (ITAMS) of CD3ζ transduces downstream cytotoxic signaling cascades in a standard CAR, similar to that of the TCR. CD3ζ phosphorylation ultimately results in the secretion of perforin, granzyme B, and other cytokines that induce apoptosis in targeted cells and inflammatory responses in the endogenous immune system. Co-stimulatory domains (**Fig.1E**) , such as CD28 or 4-1BB (CD137), which are currently included in all FDA approved CAR T products, have been shown to augment effector function of CAR T cells by increasing cytokine production, T cell proliferation, and potentiate anti-tumor activity over CAR T cells lacking co-stimulation (Finney et al., 2004). These co-stimulatory domains differ in benefits; CD28 promotes initial rapid tumor clearance, while 41BB may favor long-term persistence (Cappell and Kochenderfer, 2021). Other co-stimulatory domains are actively being investigated including OX40 (CD134), ICOS, and CD27 (Abate-Daga and Davila, 2016; Finney et al., 1998).

Multiple iterations of CAR structures have been investigated for therapeutic efficacy leading to multiple generations of CAR design. All CAR T cell generations include the antigen recognition, hinge, transmembrane, and intracellular signaling domains. The first-generation CAR consisted of only a singular signaling domain with no co-stimulatory support. This single activation mechanism had limited cytokine production and poor overall effector performance. Second-generation CARs introduced a single co-stimulatory domain to the construct. All currently FDA approved CAR T cell therapies are derived from second generation CARs.

It was hypothesized that multiple co-stimulatory domains could provide further improvement to CAR T cell therapy, so the third-generation CAR incorporates two or more co-stimulatory domains.

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Unfortunately, the third-generation CAR failed to significantly outperform the second generation (Morgan et al., 2010; Till et al., 2012), so all further iterations have been modified from the second-generation structure. The fourth-generation CAR includes the addition of an inducible or constitutive transgenic protein or cytokine to support T cell effector function by favoring T cell maintenance and survival. Some examples of cytokines expressed in fourth-generation CARs include, but are not limited to, IL-15, IL-2, and IL-7 which are necessary activation and expansion cytokines (Chmielewski and Abken, 2015; Krenciute et al., 2017). A more recent example of a fourth-generation CAR design is the constitutive over-expression of 41BBL on the surface of CAR T cells (Zhao et al., 2015). 41BBL binds the 41BB receptor in cis and provides additional CAR T cell sustainability support (Nguyen et al., 2020).

As illustrated, CARs can differ substantially in structural design spanning from the antigen being targeted to the intricate network of the signaling domain. CAR design may influence the efficacy at which tumors are targeted and is an important consideration as the field expands to combinatorial approaches. Taking this into consideration, throughout this review, we will highlight the antigen being targeted as well as the co-stimulatory domain(s) expressed (denoted as Target.Costim).

Collectively, there is substantial preclinical and clinical evidence supporting CAR T cell immunotherapy for cancer treatment. However, there are significant challenges that have hindered the holistic treatment success of CAR T cells against cancer. Established challenges of CAR T cells include properties of the tumor itself (limited identification and sustainability of novel antigens, aggressive tumor burden, hostile immune microenvironment) and CAR T cell intrinsic deficiencies (unsuccessful homing and tumor infiltration, minimal persistence over time). **(Fig. 2)**

III. Addressing Challenges of CAR T Cell Therapy with Combinatorial Chemotherapies

The challenges faced by CAR T cell therapy have hindered the success of clinical trials and ultimately the number of FDA approvals, especially for solid and brain tumors where none have been approved to date. Many groups are addressing CAR T cell intrinsic deficiencies by introducing secondary genetic modifications that improve CAR T cell efficacy. Some examples include genetic

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knockdown of negative regulators such as PD-1 (Rupp et al., 2017), CTLA4 (Zhang et al., 2019), and LAG3 (Zhang et al., 2017b), and negative epigenetic regulators such as DNMT3A (Prinzing et al., 2021), TET2 (Jain et al., 2023), SUV39H1 (Jain et al., 2024; López-Cobo et al., 2024). An alternate strategy is expression of positive signaling molecules such as C-Jun (Lynn et al., 2019) or RUNX3 (Tang et al., 2023; Zhu et al., 2023), or constitutively active cytokine receptors (Bell et al., 2023). Secondary modifications have had instances of improving CAR T cell fitness/persistence and tumor infiltration, but other tumor-driven challenges such as antigen dilemma/tumor heterogeneity, tumor burden, and the hostile tumor microenvironment are challenging to address using these approaches (Rafiq et al., 2020). Additionally, secondary genetic modifications require thorough preclinical efficacy and safety testing, which is time consuming and expensive, making it slow to implement in the clinic. However, adding an already FDA approved chemotherapeutic to a cancer regimen in combination with CAR T cells is much more readily achieved. In addition to chemotherapies, multiple other avenues have been investigated to support the anti-tumor activity of CAR T cells. For example, investigators have sought to increase the persistence and potency of CAR T cells by combining them with supportive cytokines, interferons, and antibodies. Although noteworthy, we have narrowed the scope of this review to studies focused on combination treatments with CAR T cells and chemotherapeutics.

To the best of our knowledge, **Table 1** represents a comprehensive list of all preclinical investigations combining CAR T cells with chemotherapy. Our thorough literature review/search found 132 unique preclinical CAR T cells and chemotherapeutic combination studies involving 67 different compounds (**Table 1**). Studies are grouped based on the CAR T cell limitation that was addressed with combinatorial therapy. For more information, **Supp. Table 1** further elaborates on the mechanisms/primary findings for each publication. In the remainder of this review, we highlight some, but not all, of the published studies that explore the ability of chemotherapeutics to be used in combination with CAR T cells across multiple cancer types with varying CAR T cell targets. We highlight examples where each of the challenges faced by CAR T cells can be addressed by a combinatorial

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approach. We further provide mechanistic insight into how the chemotherapeutic augmented CAR T cell efficacy and how it may be more broadly investigated.

A. Antigen Dilemma/Tumor Heterogeneity

When considering CAR T cell therapy for a given cancer, the first challenge is to identify a targetable tumor antigen (Wei et al., 2019).To be a viable target, the tumor antigen is ideally a surface-anchored protein that us highly expressed on tumor cells for sufficient CAR T cell activation, not expressed on surrounding or distal healthy tissues, and not expressed on the CAR T cells themselves or any immune cells (Abbott et al., 2020; Breman et al., 2018; Wei et al., 2019). Expression on healthy tissues leads to on-target off-tumor toxicity that can be harmful to a patient (Castellarin et al., 2020; Flugel et al., 2023). Expression on CAR T cells leads to fratricide, or self-killing, that diminishes the potency of the therapy against the cancer (Breman et al., 2018). Even once a target is identified, many tumors are heterogenous and not all cell populations may express the same surface antigen or at the same level, leading to incomplete tumor eradication. Different tumor types can regulate antigen expression in a variety of ways that may hinder CAR T cell efficacy. Broadly, the term “antigen dilemma” defines tumor associated antigens that have low basal expression, are heterogenous in nature, may downregulate in response to treatment, or are indiscriminate on tissue expression leading to off-target toxicities. To combat this limitation, it is desirous to identify chemotherapeutics that can modulate the expression of tumor antigens. Throughout this review, we will denote which branch of the antigen dilemma is being targeted by the different compounds. A complete list of compounds that address the antigen dilemma is found in **Table 1** and **Supp. Table 1**.

One way to accomplish this is to increase the expression of an antigen that is already present on tumor cells. In 2018, Jetani et. al. published the first example of this with FLT3.CD28 CAR T cells targeting acute myeloid leukemia (AML) (Jetani et al., 2018). FKT3.CD28 CAR T cell monotherapy had potent but limited anti-tumor efficacy against FLT3^{+} AML cells lines *in vivo*. Treatment with the preclinical FLT3 inhibitor crenolanib increased the surface expression of FLT3 on AML blasts and

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sensitized them further to FLT3.CD28 CAR T cell treatment. However, healthy hematopoietic stem cells (HSCs) express FLT3 and were found to be susceptible to elimination following FLT3.CD28 CAR T cell administration. Crenolanib did not increase FLT3 expression on HSCs, but all healthy HSCs were eliminated *in vivo* upon CAR treatment (Jetani et al., 2018). The safety of this strategy would require CAR T cell elimination after tumor clearance and re-engraftment of HSCs. HSC transplantation is very common for treating hematological malignancies in the clinic and would not present a significant roadblock to clinical translation of this combinatorial approach (Hasan et al., 2024).

Similarly, ALK1 has been identified as a promising immunotherapeutic target for neuroblastoma (NB) as it is upregulated on the surface of tumor cells but not on healthy tissues and has oncogenic pathology (Carpenter and Mossé, 2012; Wulf et al., 2021). Bergaggio et. al. developed the first human and murine ALK.CD28 CAR T cells in 2023 that effectively recognize and eliminate NB tumors highly expressing ALK (Bergaggio et al., 2023). However, ALK.CD28 CAR T cells largely failed to elicit responses in preclinical models of low ALK expressing cell lines. Previous literature has shown that treatment of NB with ALK inhibitors such as the FDA approved agent lorlatinib have promising yet limited efficacy as a monotherapy (Goldsmith et al., 2023). However, one consequence of lorlatinib exposure is stabilization of ALK surface expression, presenting a unique opportunity for combinatorial therapy with CAR T cells. In NB lines with low ALK expression, combinatorial therapy with lorlatinib and ALK.CD28 CAR T cells significantly extended survival over ALK.CD28 CAR T cell monotherapy in multiple preclinical models (Bergaggio et al., 2023). This is a promising strategy for NB as all NB patients with high or low initial ALK expression could benefit from combinatorial treatment with lorlatinib through surface stabilization of the target antigen and innate anti-tumor activity of lorlatinib. ALK alterations and overexpression have also been shown in colorectal cancer and metastatic non-small cell lung carcinoma (Zhao et al., 2023), suggesting this combinatorial approach could be further explored in other cancer types.

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A more challenging strategy is to consider inducing expression of a tumor antigen that is not naturally expressed on tumor cells. In a recent example, Harrer et. al. optimized a dosing regimen with the FDA approved therapeutic decitabine and saw expression of the CAR target CSPG4 on the surface of ovarian carcinoma cells that were negative before treatment (Harrer et al., 2022). Treatment with CGSP4.CD28 CAR T cells was only effective against decitabine treated ovarian carcinoma cells, representing the first published chemotherapy-induced antigen expression with subsequent antigen-specific targeting. While promising, this approach was not tested *in vivo* and requires further preclinical optimization before considering clinical implications. Decitabine and structural variant azacitidine has also been shown to upregulate antigen expression of CD70, CD123, and CD19 in various hematological malignancies, highlighting the immense potential of this class of small molecules to modulate antigens in combinatorial CAR T cell therapy (El Khawanky et al., 2021; Leick et al., 2022).

B. Tumor Burden

The next challenge for CAR T cell therapy to overcome is the burden (ex. size, aggressive growth) of target tumors. CAR T cell clinical trials most often only enroll patients that are relapsed or refractory to standard treatment and tumors are substantial in size and growth rate. An effective CAR T cell therapy needs to be able to tackle a large and aggressive tumor. While CAR T cells are effective killers, therapy benefits significantly from combinatorial approaches that can de-bulk tumors or slow their growth prior to or during CAR T cell administration. A complete list of compounds that address tumor burden is found in **Table 1** and **Supp. Table 1**.

A simple example of this approach would be to treat tumors with chemotherapeutics that have known anti-tumor efficacy along with CAR T cells so that both therapies are independently yet synergistically working to clear the tumor. A preclinical study by Zhang et. al. in colorectal cancer models evaluated the efficacy of combinatorial efficacy of the kinase inhibitor regorafenib and EpCAM-CAR natural killer (NK) cells (Zhang et al., 2018b). Regorafenib and EpCAM CAR-NKs had anti-tumor efficacy alone, but combination significantly reduced the rate of tumor growth over either monotherapy

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in preclinical models. While not yet published preclinically, one Phase 1 clinical trial has reported findings from 6 initial patients receiving GPC3.41BB CAR T cells for treatment of hepatocellular carcinoma (Fu et al., 2023). Of the 6, 4 patients received combinatorial treatment with sorafenib, a similar kinase inhibitor, during CAR T cell infusions. The data is not yet suggestive of an added or reduced benefit, but clinical testing of CAR T/kinase inhibitor combinatorial strategies will shed more light on future viability of this combination.

In another example, chemotherapeutic treatment with the FDA approved chemotherapy cisplatin is the first line of defense for gastric cancer. While cisplatin very effectively clears away more differentiated tumor cell populations, stem cell tumor populations are resistant to cisplatin and readily lead to tumor recurrence and metastasis. Published studies have shown that these stem cells are positive for CD133 and that cisplatin treatment increases CD133 expression on stem cells. Han et. al. developed a CD133.CD28 CAR T product that was preclinically tested in combination with cisplatin treatment against gastric cancer (Han et al., 2021). Compared to either monotherapy, combination therapy resulted in decreased tumor burden in xenograft models, specifically with reduced stem cell populations in any remaining or relapsed tumor due to CD133 CAR efficacy. This strategy highlights the ability of CAR T cells and chemotherapy to be mutually beneficial – cisplatin diminishes the tumor size allowing CAR T cells to have less bulk to clear, and CAR T cells eliminate the tumor cells that are resistant to cisplatin treatment.

Another way to tackle aggressive tumor growth is to sensitize tumor cells to CAR T cell therapy, rendering it more effective. In one example, pre-treatment of leukemia models with indometacin sensitized cancer cells to CD19 CAR T cell therapy (Aboelella et al., 2022; Naval et al., 2019). Specifically, indometacin treatment increased the surface expression of death receptor 5 on tumor cells. TNF-related apoptosis induing ligand (TRAIL) is an endogenous ligand for death receptor 5 that can be secreted by activated T cells to assist in tumor cell cytotoxicity (Naval et al., 2019). In this system, indometacin induced death receptor 5 expression on tumor cells to sensitize them further to CAR T cell

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cytotoxicity through both CAR recognition and TRAIL mediated apoptosis. This strategy could be broadly applied to many cancers, as the DR5/TRAIL signaling axis can be activated on all tumor cells.

C. CAR T Cell Infiltration

Successful trafficking of CAR T cells to tumor sites, especially solid tumors, can be significantly hindered by the physical and physiologic conditions of the tumor (Scharping et al., 2016). The restrictive nature of solid tumors to infiltrating lymphocytes is due in part to the fibrous, dense, and rigid nature of the extracellular matrix (Henke et al., 2019; Salmon et al., 2012). Additionally, dysregulated tumor vasculature complicates the penetration and motility of CAR T cells to and through the tumor stroma (Lanitis et al., 2015; Park et al., 2023). Furthermore, many tumor types are characterized by a large degree of cellular heterogeneity that complicates successful tumor recognition and infiltration. Therefore, chemical compounds that can modulate tumor or CAR T cell properties to potentiate homing and infiltration are desirous. A complete list of compounds that address CAR T cell infiltration is found in **Table 1** and **Supp. Table 1**.

The DNA damaging chemotherapy carboplatin has shown promising results in mitigating the dense extracellular matrix (ECM) of solid tumors. In a recent study by Porter. et. al., administration of carboplatin increased the frequency of cancer associated fibroblasts (CAFs) in a model of prostate cancer (Porter et al., 2023). Firstly, CAFs underwent a pro-inflammatory shift, encouraging and assisting CAR T cell infiltration. Secondly, carboplatin treated CAFs had increased expression of multiple ECM degrading matrix metalloproteinases (*Mmp2, Mmp3, Mmp13, Mmp 14, and Mmp27*). When treated in combination with carboplatin, Le^{y}.CD28 CAR T cells had greater infiltration, accumulation, and cytotoxic potential against multiple prostate patient derived xenografts (Porter et al., 2023). Other platinum-based therapies have also been shown to increase CAR T cell infiltration. Oxaliplatin was able to increase the infiltration and cytotoxic effects of ROR1.41BB CAR T cells in a murine lung adenocarcinoma model. Oxaliplatin mediated immune landscape remodeling by inducing tumor associated macrophages to express T-cell recruiting chemokines (*Cxcl9 and Cxcl10*). The resulting increased infiltration of

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ROR1.41BB CAR T cells served as a positive feedback loop as IFNγ secretion by CAR T cells mediated more production of chemokines by the macrophages (Srivastava et al., 2021).

The hypoxic nature of tumors can further limit CAR T efficacy by diminishing CAR T cell fitness and tumor cell antigen expression leading to decreased homing and recruitment potential (Berahovich et al., 2019; Li et al., 2020; Sethumadhavan et al., 2017). A 2020 study by Li et al demonstrated that combinatorial therapy of CAIX.41BB CAR T cells with sunitinib, an inhibitor of the receptor for tyrosine kinase, resulted in improved infiltration of CAR T cells into tumor tissue in a model of renal carcinoma (Li et al., 2020). This was true under both normoxic and hypoxic conditions and was dose-dependent in relation to sunitinib. Sunitinib ultimately increased CAIX antigen expression on tumor cells which allowed for better homing of CAR T cells to the tumor site. This showcases another way in which increasing antigen density on tumor cells promotes CAR T cell activity. Interestingly, improved infiltration due to CAIX.41BB CAR T cell combination with sunitinib in the renal carcinoma model did not produce a therapeutic benefit. However, there was a combinatorial therapeutic benefit in a metastatic lung model, illustrating heterogeneity in response and that lack of response in one tumor model does not discount the potential utility of the approach in others. Instead, it suggests that infiltration was not the primary hinderance to CAR T cell efficacy in the renal carcinoma model. Of note, combined treatment of CAIX.41BB CAR T cells with sunitinib reduced the amount of immunosuppressive myeloid derived suppressor cells (MDSCs) at the tumor site (Li et al., 2020). This is a common theme surrounding many of the compounds that increase the homing and infiltration of CAR T cells - they tend to also alter the tumor immune cell landscape either directly or indirectly.

D. Hostile Tumor Microenvironment

The immune system can significantly influence the efficacy of numerous therapies used to treat cancer, including immune and CAR T cell therapies (Johnson et al., 2022). For CAR T cells, hostility is largely driven by the immunosuppressive cells within the tumor that promote tumor immune escape during oncogenesis. The primary immunosuppressive cells include T regulatory (Treg) cells, MDSCs,

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and tumor associated macrophages (TAMs) (Quail and Joyce, 2013). Tregs suppress T cell effector function and activation through secretion of immunosuppressive cytokines, competition for cytokines CAR T cells need to survive, and downregulation of stimulatory antigens on antigen presenting cells (Thornton and Shevach, 1998; Wing et al., 2008). MDSCs have been shown to specifically inhibit CAR T cell function through a host of mechanisms including Treg induction and stimulation, nutrient depletion, reactive oxygen species production, and anti-inflammatory cytokine secretion (Huang et al., 2006; Markowitz et al., 2017; Raber et al., 2014; Srivastava et al., 2010; Yu et al., 2013). TAMs contribute to immunosuppression by increasing the expression of amino acid depleting enzymes such as indoleamine 2,3- dioxygenase (IDO1), secreting immunosuppressive cytokines, and recruiting Tregs (Wang et al., 2019; Yan et al., 2019; Yan et al., 2015; Ye et al., 2018). Of importance, TAMs are often the most abundant tumor infiltrating immune cells, highlighting their grave importance in CAR T cell-based immunotherapy (Bied et al., 2023; He and Zhang, 2021). Chemotherapies that can mitigate immunosuppression in the context of CAR T cell therapy are extremely beneficial to the field and many studies have illustrated the ability of chemotherapies to combat this challenge. A complete list of compounds that address the hostile tumor microenvironment is found in **Table 1** and **Supp. Table 1**.

A study led by Xia et al utilized genomics and transcriptomics to reveal that the epigenetic modulator, BRD4, mediates immunosuppression driven resistance of glioblastoma (GBM) cell lines to EGFR.CD28.41BB CAR T cells. Treatment with the BET inhibitor, JQ1, in combination with EGFR.CD28.41BB CAR T cells was able to suppress the induction of immunosuppressive proteins IL-6, IL-8, IDO-1, and programmed death ligand 1 (PDL-1). This combination was also able to extend survival of the mice and prevent metastasis more significantly than either treatment alone (Xia et al., 2021a). CAR T therapy in GBM has also benefited from combined treatment with the lactate generation inhibitor, oxamate, to overcome the immunosuppressive microenvironment. Excess lactate production is a byproduct of aberrant glycolytic activity in tumor cells which contributes to the increased expression of ATP converting ectonucleotidases, CD39 and CD73. CD39 and CD73 scavenge pro-inflammatory

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molecules to generate immunosuppressive byproducts. Oxamate treatment was shown to decrease the CD39 expression in TAMs and Treg cells supporting a less hostile tumor microenvironment that allows for greater anti-tumor activity of EGFRvIII.41BB CAR T cells (Sun et al., 2023).

Another compound investigated for immune-modulatory roles to enhance the efficacy of CAR T cells is the anti-neoplastic agent docetaxel. Docetaxel combination therapy was tested in a xenograft model of prostate cancer in combination with prostate specific membrane antigen (PSMA) CAR T cells. The combination of docetaxel and PSMA.41BB CAR T cells was shown to decrease tumor burden and increase survival probabilities *in vivo.* Mechanistically, this combination ultimately reduced the ratio of immunosuppressive MDSCs to CAR T cells, providing a less immunosuppressive environment (Zhang et al., 2022).

As technology advances there are now novel approaches to administer immune landscape altering compounds. Shao et al revealed that nanosheets loaded with the IDO-1 inhibitor, epacadostat, supported anti-tumor activity CD19.CD28 CAR T cell therapy in esophageal squamous cell carcinoma. Epacadostat loaded nanosheets reduced IDO-1 facilitated production of the immunosuppressive metabolite kynurenine, supporting a more permissive tumor microenvironment to the CAR T cells. *In vivo* tissue analysis revealed that mice treated with CD19.CD28 CAR T cells and epacadostat loaded nanosheets had lower expression of exhaustion markers (PD-1 and TIM3) and increased effector cytokines (IL-2, IFNγ, and Perforin) (Shao et al., 2021). In a 2018 study, Zhang et al generated nanoparticles loaded with either the PI3K inhibitor PI-3065, to regulate Treg subsets, or 7DW8-5, a stimulatory agonist of effector cells. Using EGFRvIII.CD28 and ROR1.CD28 CAR T cells against breast cancer cell lines, the combination therapy with nanoparticles enhanced cytotoxicity and persistence of CAR T cells supporting improved survival (Zhang et al., 2018a).

While targeting single populations within the tumor immune microenvironment has been beneficial, it is possible that other immunosuppressive mechanisms might compensate when one is eliminated, ultimately dampening the strength of a combinatorial approach. One unique way to

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holistically target the immunosuppressive compartment is to consider multiple immune targeting compounds. A resulting "polypharmacy" approach was tested by Sullivan et. al. in a rhabdomyosarcoma model in combination with FGFR CAR T cells. The treatment schema involved FGFR CAR T cells along with antagonists to CSF1R, IDO1, iNOS, TGFβ, and PD-L1. The "polypharmacy" and CAR T cell combinatorial approach successfully controlled tumor burden and increased survival in an orthotopic model of rhabdomyosarcoma (Sullivan et al., 2022). While this is encouraging, the administration of several compounds comes with more potential for unwanted toxicities and safety evaluation must be performed with extreme diligence.

One final important consideration for chemo and CAR T cell combination therapy and the influence of the immunosuppressive microenvironment is the administration of lymphodepleting agents. Commonly, a combined regimen of cyclophosphamide and fludarabine are used as a lymphodepleting cocktail to promote a stable environment for CAR T cells to establish and persist in hematological malignancies and has been heavily reviewed elsewhere (Amini et al., 2022; Lickefett et al., 2023; Ramos et al., 2018). Some solid and brain tumor trials are also incorporating lymphodepletion prior to CAR T cell therapy as ways of removing immunosuppressive B-cells and Tregs from the tumor microenvironment (Heczey et al., 2017; Suryadevara et al., 2018). While lymphodepletion is often considered common practice, it is important to understand the interplay between lymphodepleting agents and a lymphoid-based (CAR T) cell therapy. A 2022 clinical study led by Fabrizio et al illustrated the importance of determining optimal fludarabine concentrations in patients with B cell acute lymphoblastic leukemia (B-ALL) undergoing CD-19 CAR T cell therapy. Patients in this cohort treated with suboptimal concentration of fludarabine had higher risks of relapse (Fabrizio et al., 2022). Therefore, as the field of CAR T cell therapy progresses, lymphodepleting schemas must be diligently designed to ensure that CAR T cells have the best conditions to initiate cytotoxic potential. Furthermore, the interactions of any combinatorial chemotherapeutics with lymphodepleting agents must be considered to devise appropriate treatment strategies that provide optimal therapeutic windows.

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E. CAR T Cell Fitness

An additional challenge faced by all CAR T cell therapies is the limitation of anti-tumor activity caused by the inability of CAR T cells to persist in a functional effector state. Loss of functionality upon antigen exposure is called exhaustion and is marked by an increase in inhibitory receptors on the CAR T cell surface such as PD-1 and CTLA4. Monoclonal antibody blockade of these signaling axes has been widely employed in the clinic (Wei et al., 2018) to re-invigorate the population of CAR T and endogenous T cells that have successfully infiltrated a tumor but have become exhausted (Korman et al., 2022; Makuku et al., 2021). Additionally, many secondary CAR T cell genetic alterations aim to improve the overall fitness (persistence, metabolism, effector function, etc.) of tumor-infiltrating CAR T cells. To maintain fitness, CAR T cells need to be able to resist exhaustion (Chow et al., 2022), metabolically compete for limited nutrients in the tumor microenvironment (Peng et al., 2023), and form long-lasting memory subsets that can clear primary tumors and prevent relapse (Doan et al., 2024). There are many targetable pathways involved in these processes, as evidenced in **Table 1** and **Supp. Table 1** where the majority of CAR T cell and chemotherapy combinatorial studies have focused on CAR T cell fitness. Dosing may be accomplished in 2 fundamental ways. Firstly, chemotherapeutics can be added to the CAR T cell manufacturing process to influence the pathways and properties of CAR T cells prior to infusion. Secondly, chemotherapies can be administered to patients during or after CAR T cell infusion to influence the fitness of CAR T cells within the tumor microenvironment. Herein, we review examples of both strategies.

1. Chemotherapies Added During the Manufacturing Process

Chemotherapies that can positively influence CAR T cells via addition in the manufacturing process are highly desirable as this approach can be broadly applied to any cancer therapy schema. A prime example of adding chemotherapeutics to the manufacturing process is the addition of the Src kinase inhibitor, dasatinib. Dasatinib incubation keeps CAR T cells in a rested “off” state that enriches naïve or stem-like phenotypes prior to infusion (Mestermann et al., 2019; Watanabe et al., 2023; Weber

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et al., 2019; Weber et al., 2021). Evidence suggests that a high proportion of stem-like cells in CAR T cell infusion products may correlate with strong anti-tumor efficacy. Dasatinib is commonly used in clinical and preclinical CAR T cell manufacturing processes for this reason. Similarly, combination therapy of GD2.CD28.41BB with IGF1R/IR inhibitor linsitinib against diffuse intrinsic pontine glioma (DIPG) improved therapeutic efficacy by maintaining CAR T cells in a more un-differentiated central memory state (de Billy et al., 2021). This led to improved therapeutic efficacy at lower CAR T cell doses compared to CAR T cell treatment without linsitinib pretreatment. This result is very encouraging as DIPGs are highly aggressive pediatric brain tumors that have no cure (Farrukh et al., 2023).

Previously, we discussed that the FDA approved therapeutic decitabine can induce the surface expression of antigens on cancer cells and may address the CAR T cell antigen dilemma (Harrer et al., 2022). When added to the CAR T cell manufacturing process, decitabine alters the epigenetic landscape of CAR T cells to improve fitness by favoring memory formation and persistence (Wang et al., 2021). CD19.41BB CAR T cells pretreated with decitabine successfully eliminated large tumor burdens in preclinical models of ALL and prevented tumor growth upon rechallenge. This highlights the multi-functionality of a chemotherapeutic like decitabine – when administered to a tumor it can increase surface antigens and when administered only to T cells it improves fitness.

CAR T cell fitness is commonly impaired by the metabolic hostility of the tumor microenvironment. Tumor cells and components of the tumor immune microenvironment out-compete CAR T cells for essential nutrients. In 2024, Si et al published a study evaluating the impact of pretreating CAR T cells with the FDA approved chemotherapeutic enasidenib, an isocitrate dehydrogenase 2 (IDH2) inhibitor (Si et al., 2024). They found that IDH2 genetic ablation or inhibition with enasidenib diverted glucose utilization away from glycolysis towards the pentose phosphate pathway which improved activity under nutrient-starved conditions. Furthermore, central memory formation was enriched due to the shuttling of citrate into the cytosol for acetyl-CoA conversion that altered the epigenetic landscape of CAR T cells, similar to that seen by decitabine pretreatment (Wang

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et al., 2021). Ultimately, metabolically reprogrammed (via enasidenib pretreatment) CD19.41BB and GD2.CD28 CAR T cells had superior anti-tumor activity against ALL and osteosarcoma preclinical models, respectively. Importantly, anti-tumor efficacy was further improved by daily oral administration of enasidenib (Si et al., 2024). This showcases that prolonged CAR T cell support may be necessary beyond the manufacturing process for a chemotherapeutic combination strategy to be optimal.

2. Chemotherapies Administered with or Post CAR T Cell Infusion

In 2016, Ruella et. al. published a study evaluating CD19.41BB CAR T cells with combinatorial ibrutinib against mantle cell lymphoma (MCL) (Ruella et al., 2016). Animals received CAR T cell therapy on day 7 post-tumor engraftment and daily administration of ibrutinib for the duration of the study. Combinatorial treatment resulted in long-term remission that neither monotherapy could achieve. Ibrutinib is known to inhibit TH2 polarization (less cytotoxic) of T cells and favor TH1 polarization (more cytotoxic), favoring effector function (Mhibik et al., 2019). This study concurrently showed downregulation of T cell exhaustion markers PD-1 and CTLA4, overall improving the fitness of CAR T cells with combinatorial therapy (Ruella et al., 2016). Importantly, ibrutinib has also been shown to have a positive impact on CAR T cells in the clinic. Fraietta et. al. published a clinical study examining the functionality of CD19.41BB CAR T cells that were generated from chronic lymphocytic leukemia (CLL) patients that were treated with the BTK inhibitor ibrutinib (Fraietta et al., 2016). CLL is a B-cell malignancy that is susceptible to CAR T cell therapy. However, T cells from CLL patients poorly expand *ex vivo* and complicate the CAR T cell manufacturing process. In the 2016 report, the authors found that CAR T cells generated from patients with prolonged ibrutinib treatment experienced significantly improved CAR T cell expansion. Improvements in CAR T cell expansion with prolonged ibrutinib treatment correlated with improved patient response to CAR T cell therapy and overall survival (Fraietta et al., 2016). The positive impact ibrutinib had on patient T cells in the clinic emphasizes the immense potential for use in combinatorial treatment strategies to improve fitness.

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In another example, combinatorial treatment of CD19 and CD123 CAR T cells with the preclinical BET inhibitor JQ1 improved CAR T cell fitness via reduction of CAR T cell exhaustive markers, ultimately increasing AML tumor control in mouse models (Zhong et al., 2022) The mechanism was two-fold: JQ1 treatment prevented or reversed the exhaustive phenotype of CAR T cells marked by PD-1 and TIM-3 expression and diminished the level of PD-L1 expression on AML blasts. The PD-1/PD-L1 inhibitory axis relies on PD-1 on T cells recognizing PD-L1 on tumor cells. Depletion of both sides of the axis reduces the inhibitory signals received by CAR T cells and improves activation and fitness. This study highlights the ability of a chemotherapeutic to have both activating impact on CAR T cells and simultaneous inhibitory effects of cancer cells that ultimately improve CAR T cell fitness.

F. Multiple Action Chemotherapies

So far, we have highlighted chemotherapy combination strategies that are primarily single acting and modulate only one aspect of either CAR T or tumor biology but also some that have multiple mechanisms of action. For example, in a prostate cancer model using Le^{Y}·CD28 CAR T cells, carboplatin induced both ECM remodeling of the tumor and promoted anti-tumorigenic macrophage polarization, conferring survival benefit for combination treated mice (Porter et al., 2023). We view these multi-action combinations to be highly valuable, especially if providing a positive benefit to CAR T cells while simultaneously negatively impacting tumor growth and survival.

A commonly used chemotherapeutic for CAR T cell combination therapy both preclinically and clinically is lenalidomide (Kann et al., 2024; Thieblemont et al., 2020; Wang et al., 2020; Zarei et al., 2023). Lenalidomide has been tested against multiple hematological and some solid malignancies in combination with BCMA, CD19, CD20, CD23, CD133, CS1, HER2, NKG2D, and WT-1 targeting CAR T cells (**Table 1**). Lenalidomide is standard for multiple myeloma (MM) therapy, allowing for direct anti-tumor activity (Holstein et al., 2018). In combinatorial treatment with CAR T cells, lenalidomide was found to improve expansion of CAR T cells *in vivo* (Kann et al., 2024), improve CAR T cell effector functions (Zarei et al., 2023), and prevent early onset of CAR T cell exhaustion (Works et al., 2019).

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Lenalidomide has also been cited to increase IFNγ and IL-2 production, reduce angiogenesis (Jin et al., 2023), improve the CAR T cell/tumor cell interaction (Tettamanti et al., 2022), and improve CAR T cell tumor infiltration (Zhang et al., 2021). It is clear that lenalidomide enhances CAR T cell therapy via direct tumor and CAR T cell mechanisms and is promisingly being actively investigated in the clinic.

Another multi-action combinatorial strategy was recently published by Gao et. al. targeting sphingosine 1-phosphate receptor 3 (SIPR3) on tumor cells in both breast and colon murine cancer models (Gao et al., 2023). High expression of SIPR3 has been heavily associated with poor patient prognosis as well as failure of checkpoint blockade therapy in cancer patients. Murine EpCAM.CD28.41BB cells were combined with TY-52156 or CAY10444 SIPR3 inhibitors in co-culture with tumors and showed increased activation upon exposure to antigen (as determined by IFN and granzyme B secretion), increased memory phenotype (as determined by CD44+CD62L+ double expression), and reduced exhaustion (as determined by PD-1, TIM-3, and LAG-3). Combination was more efficacious in controlling tumors *in vivo* than either monotherapy alone. This was further attributed to the ability of SIPR3 inhibitors to reprogram the tumor immune microenvironment by polarizing macrophages towards the M1 proinflammatory phenotype (Gao et al., 2023). M1 macrophages are not suppressive for CAR T cells and enable them to continue to effectively clear tumor cells (Rodriguez-Garcia et al., 2021). SIPR3 inhibitors are a prime example of chemotherapeutics with multiple mechanisms of action that synergize to improve tumor clearance. Additionally, it is a strategy that could likely be applied to any cancer with high SIPR3 expression.

These multiple action drugs, in our opinion, should be prioritized as cancer researchers appreciate and try to combat the immense complexities of cancer. Targeting different cancer associated phenotypes with a single compound may minimize disease burden, treatment-related cytotoxicity, and support an environment that allows for the highest cytotoxic potential of CAR T cells.

IV. CAR T Cells That Address Chemotherapeutic Limitations

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The tumor targeting capability of CAR T cells presents a unique opportunity to further overcome some limitations of chemotherapeutics. Engineering CAR T cells to behave like nanoparticles would allow for local administration of a secondary therapeutic alongside CAR T cell cytotoxicity. To date, this has been most explored in the context of CAR T cells that locally deliver monoclonal checkpoint blockade therapies which are antibodies that block inhibitory signaling axes between tumor cells and CAR cells (ex. PD-1/PD-L1, CTLA4/CD80(86)) (Rafiq et al., 2018). CAR T cells have also been designed to secrete enzymes to modulate the tumor microenvironment. In one study, GD2.CD28.OX40 CAR T cells that secrete heparinase induced ECM degradation and improved infiltration of CAR T cells into solid tumors (Caruana et al., 2015).

Gardner et. al. moved this concept into the field of chemotherapeutics and developed 'SEAKER' (synthetic enzyme-armed killer) CAR T cells in 2021 that secrete enzymes capable of cleaving inactive prodrugs into their active counterparts, inducing a combinatorial therapy only at the site of CAR T cell localization (Gardner et al., 2022). Specifically, SEAKER cells deploy enzymes derived from bacteria (CPG2 and β-lac) that would be active only on the prodrug containing the enzymatic recognition site and be innocuous to healthy tissues. The secretable enzyme sequence was co-transduced with a CD19.41BB CAR. SEAKER cells effectively activated the chemotherapeutics 5′-*O*-Sulfamoyladenosine (AMS), the nitrogen mustard ZD2767, and 7-*O*-aminopropyl-7-*O*-des(morpholinopropyl)gefitinib (APdMG) when masked with either glutamate (cleaved by CPG2) or cephalosporin (cleaved by β-lac). Combinatorial treatment with SEAKER cells and respective prodrugs reduced tumor burden and extended survival against hematological and solid malignancies. Notably, SEAKER cells were able to eliminate antigen negative cancer cells *in vitro* in the presence of appropriate prodrug where CAR T cells alone could not due to low-level secretion of enzymes that is not antigen dependent. This strategy therefore also addresses some antigen dilemma/heterogeneity challenges where local activation of potent chemotherapeutics can eliminate any tumor cells that CAR T cells cannot target due to low or absent antigen expression.

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The modularity of SEAKER technology is exciting for the development of the next waves of combinatorial treatment options for CAR T cells and chemotherapeutics. Prodrugs are desirable for their ability to be specifically activated at the tumor site, limiting off-tumor toxicities (Markovic et al., 2020; Rautio et al., 2008). Prodrugs can be designed with hypoxia or acid-sensitive caps, as well as enzyme cleavable moieties (Markovic et al., 2020). The appeal of the SEAKER cell technology is the incorporation of bacterial derived enzyme cleavage linkers that cannot be cleaved unless SEAKER cells are present. This provides the possibility of conjugating cytotoxic drugs with tumor targeting moieties or blood brain penetrant moieties (Xia et al., 2021b) that can shuttle drugs to the tumor where they will be activated by SEAKER cells. This may drastically expand the number of drugs that could be considered for brain cancer therapy that currently are limited by low blood-brain barrier penetrance.

V. Identifying Chemotherapy and CAR T Combinations with High Throughput Screening

As exemplified throughout this review, combining CAR T cell therapy with chemotherapies can be highly beneficial. Many investigators have prioritized compounds based on their known mechanisms of action that either impinge on tumor cell maintenance or positively regulate CAR T cell effector functionality. However, identifying candidates can be laborious and time-consuming, delaying potential clinical translation. High throughput drug screening is an essential technique that can be optimized to streamline the drug identification process. Some successful preclinical CAR T cell and drug combinations reviewed herein have been identified using screening technology. Screens spanned small molecule inhibitors, epigenetic modulators, pro-apoptotic molecules, kinase inhibitors, and mitochondrial targeting compounds (de Billy et al., 2021; Dufva et al., 2020; Lee et al., 2022; Si et al., 2024; Zhang et al., 2023) These drug screens identified compounds that could either modulate the immune landscape, support CAR T cell effector function and fitness, increase tumor debulking, increase targeted antigen expression or promote a more permissive environment for CAR T cells. Collectively, these screens took a broad scale approach to enhancing CAR T cell therapy that more rapidly identified promising candidates to be used in downstream validation.

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For example, one study performed by de Billy et al identified insulin like growth factor receptors/insulin receptors (IGF1R/IR) as a targetable vulnerability for diffuse midline gliomas (DMGs) through a selective kinase drug screen. This screening approach was performed by pretreating DMG cells with a singular concentration (1µM) of 42 kinase inhibitors for an hour prior to the addition of either GD2.CD28.41BB or non-transduced CAR T cells. The tumor and T cells were co-cultured in the presence or absence of drug for 24 hours at which point relative viability was determined using live cell imaging and metabolism-based assays. From the drug screen, linsitinib and BMS-754807 were identified as compounds that could inhibit tumor cell viability while sparing CAR T cells individually. Further validation confirmed a therapeutic benefit in the combination of linsitinib and GD2.CD28.41BB CAR T cells that contributed to greater anti-tumor activity in both *in vitro* and *in vivo* DMG models (de Billy et al., 2021).

A more recent example of a successful compound screen was performed by Zhang et all which utilized a small molecule compound library that identified JK-184, a hedgehog signaling pathway inhibitor, to complement B7-H3.CD28 CAR T cells against breast cancer cell lines (Zhang et al., 2023). Uniquely, this study used three distinct approaches to conduct the screen where either 1.) tumor cells were pretreated with candidate compounds for 48hrs and then CAR T cells were added 2.) tumor cells were incubated with compounds for 48hr in the absence of CAR T cells or 3.) CAR T cells were incubated with the candidate compounds for 24hrs in the absence of tumor cells. This multi-pronged approach allowed for specific characterization of drug induced effects on each cell type individually while also being able to determine a combinatorial benefit. Following identification, tumor cells and CAR T cells treated with candidate compounds were subjected to RNA sequencing and gene pathway enrichment analysis to identify differentially expressed genes that may be mechanistically contributing to the observed benefit. Later experiments confirmed that JK184 enhanced B7-H3.CD28 infiltration and reshaped the tumor milieu by inhibiting immunosuppressive myeloid populations (Zhang et al., 2023).

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A main consideration for performing a drug screen is the vast heterogeneity of cancer types that drive the deficiencies of CAR T cell therapy. For instance, many CAR T cell therapies targeting blood cancers fail to prevent relapse due to antigen downregulation or loss on the surface of cancer cells (Mishra et al., 2024). This is not often a challenge with solid and brain cancers, but poor infiltration and quick progression of exhaustion are (Marofi et al., 2021). Knowing the primary challenges CAR T cells will face against a given tumor type is crucial. Then, targeted drug screens with appropriate readouts should be performed to identify candidates for combinatorial therapy. Ideally, as more screens are performed, drug candidates will surface that are effective in combination with CAR T cells regardless of CAR construction against multiple cancers. However, as the field enters further into investigations of this nature, it is imperative to perform unique screens for each tumor type and different CAR targets and structures. Screens should additionally be designed in a way that the impact of a chemotherapeutic on both the tumor and CAR T cells can be assessed.

VI. Critical Considerations for Chemo and CAR T Cell Combination Therapy

Herein, we have shown the vast number of mechanisms by which combinatorial chemotherapy can overcome challenges faced by CAR T cell therapy. Chemotherapeutics can increase surface levels of targetable surface antigens; assist cytotoxic CAR T cells via tumor debulking; improve CAR T cell homing and penetrance into solid tumor masses; re-wire CAR T cell transcription, epigenetic, and metabolic programs to improve fitness and persistence; and combat other cells in the tumor immune microenvironment that are hostile towards CAR T cells. In some cases, a single chemotherapeutic will address multiple CAR T cell deficiencies with the ability to target programs in tumor cells or tumor associated immune cells that are deleterious and in the CAR T cells that are beneficial. Additionally, here are many instances of CAR T cell and chemotherapy combination strategies in the clinic (Al-Haideri et al., 2022). As more combination strategies are being investigated, there are many different aspects that must be considered and addressed in the preclinical stage for successful implementation in the clinic.

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A. Thorough Preclinical Efficacy *and S*afety Testing

Thorough preclinical evaluation of combinatorial CAR T and chemotherapy combination strategies is imperative for successful translation into the clinic. For many CAR T cell preclinical studies, human tumors are orthotopically implanted into immunocompromised mice prior to treatment with human CAR T cells. Immunodeficiency of mice ensures that human tumors and CAR T cells will not be rejected by the murine immune system. While this methodology confirms that human CAR T cells successfully target human tumors in a living system, true safety testing is not possible in this setting. Some CAR targets highly expressed on tumor cells can be expressed on healthy tissues (ex. GD2, Epha2) which may pose on-target off-tumor health risks (Machy et al., 2023; Zhao et al., 2021). Additionally, immunocompromised mice are lacking the full tumor immune microenvironment that may significantly change the outcome of CAR T cell therapy (Haydar et al., 2023). Similarly, chemotherapeutics must undergo rigorous safety testing to ensure that the benefit of the therapy outweighs the possible risks. Even if both therapies are proven to be safe separately, safety testing of combination therapy must still be performed due to possible alterations of safety profiles of either the chemotherapeutic or CAR T cells. It is imperative to perform preclinical safety and efficacy studies of combinatorial strategies in syngeneic models with murine tumors that mimic human pathology and CAR T cells derived from murine T lymphocytes so that safety of both chemotherapeutic and CAR T cells can be evaluated in the presence of the endogenous immune system.

B. Optimizing Delivery Approach

An additional challenge for chemotherapy and CAR T cell combinatorial approaches is identifying an appropriate dosing timeline. In the most traditional sense of a combinatory strategy, two different therapies can be administered simultaneously to a patient (**Fig.3A**). This strategy may be ideal when considering a combinatorial strategy that will most profoundly impact CAR T cells within the tumor microenvironment or for chemotherapeutics that act on both CAR T cells and a component of the tumor. However, we have discussed in this review that there is also a benefit to either priming tumors (**Fig.3B**)

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or CAR T cells (**Fig.3C**) with chemotherapeutics prior to infusion, depending on the mechanism of the chemotherapeutic.

For chemotherapeutics that exclusively target the tumor, pre-treatment of patients with chemotherapeutics prior to CAR T cell administration is a viable option. For example, chemotherapeutics that debulk tumors or increase surface antigen levels are likely to be most beneficial if administered some time before CAR T cell treatment. However, it is crucial to consider the impact this will have on the health of patient T cells. A 2019 study by Das et. al. studied the naïve populations of T cells collected from the periphery of patients at the beginning of cancer diagnosis and throughout rounds of standard chemotherapy for multiple cancers (Das et al., 2019). The naïve phenotype was singled out as proportion of naïve cells in CAR T cell infusion products have been shown to correlate to treatment efficacy. 195 pediatric cancer patients across 10 different diagnoses (including hematological and solid malignancies) were enrolled onto the study. CAR T cells were generated from peripheral blood mononuclear cells, surface phenotyping was performed to identify the proportion of naïve cells, and *ex vivo* expansion was used as the primary benchmark for assessing CAR T cell functionality/health. Generally, regardless of cancer type, CAR T cell health was superior at diagnosis as compared to following any amount of chemotherapy and was dependent on the initial proportion of naïve/central memory CAR T cells. This data highlights that the timing of blood collection for the generation of CAR T cells for patients who might receive CAR T cell therapy following standard chemotherapy needs to be carefully considered to optimize efficacy. Potential combinatorial strategies should also consider whether chemotherapy might have a negative impact on overall T cell health if administered first.

For chemotherapeutics that exclusively act on CAR T cells, adding the agent to the manufacturing process is readily approved as discussed in the “Fitness” section above. However, it is possible that for some inhibitors acting on CAR T cells, like enasidenib, that maximum therapeutic benefit is achieved with pretreatment in the manufacturing process and continued administration

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following CAR T cell infusion (Si et al., 2024). Investigations into each possibility and combinations thereof are necessary to optimize the strategy.

C. Optimizing Dosing Amounts

Another consideration for appropriate dosing strategy is the overall amount of both chemotherapeutic and CAR T cell administration that is optimal for efficacy. If truly synergistic, it is hopeful that the doses of both agents can be minimized to avoid off-tumor toxicities. It is well established that chemotherapies have intense systemic toxicities that lead to sickness while on chemotherapeutic regimens as well as long-lasting effects including increased risk of developing other cancers (Brower, 2013; Demoor-Goldschmidt and de Vathaire, 2019). When CAR T cells are activated by antigen recognition, they secrete cytokines that elicit inflammatory responses. When tumors are large and CAR T cells are working effectively, this can cause patients to experience symptoms of sickness that fall on a grading scale of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) (Morris et al., 2022). Most symptoms are readily managed through treatment with tocilizumab and/or steroids, and the chemical drug ruxolitinib has also been shown to mitigate CRS symptoms when refractory to steroids (Pan et al., 2021). Optimal synergistic combinatorial therapies of chemotherapeutics and CAR T cells may reduce the dose required of both agents, resulting in maximum efficacy with minimal side effects.

Another crucial consideration for combinatorial strategies is whether a given chemotherapeutic will impair the ability of CAR T cells to successfully engraft and proliferate in response to antigen. A prominent consideration for this is when combining CAR T cells with standard-of-care therapy. Dexamethasone is commonly used in GBM treatment schema. As such, it makes sense to combine dexamethasone and CAR T cell therapies. However, dexamethasone has been shown to critically reduce CAR T cell efficacy at high doses but remain relatively inert at lower doses (Brummer et al., 2022). This highlights the importance of optimizing both timing and dosing strategies when combining CAR T cells and chemotherapeutics.

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VII. Summary and Conclusions

Overall, combinatorial CAR T cell and chemotherapeutic treatment strategies are promising for overcoming both CAR T cell and chemotherapeutic deficiencies. These possibilities are largely unexplored for many cancer types and CAR designs, representing an open field for further investigation. While there are many aspects to optimize such as CAR structure, chemotherapeutic target, and dosing strategy, combinatorial therapies are showing early preclinical success for overcoming limitations in CAR T cell therapies to ultimately improve survival outcome for patients. Finally, implementing combinatorial treatment approach to clinic has the potential to be faster and cheaper especially if both therapeutics have been FDA approved.

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Footnotes

Author Contributions

MW, AJ, and GK contributed to the writing and editing of this work. MW and AJ had equal contributions.

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Conflict of Interest

No author has an actual or perceived conflict of interest with the contents of this article.

Data Availability

This review article contains no datasets generated or analyzed during the current study.

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Table 1: List of preclinical CAR T cell and chemotherapy combination studies

	Drug(s)	FDA Appr.	Drug Description	CAR Target(s)	Cancer Model(s)	PMID
SINGLE-ACTION DRUGS (grouped by CAR T cell challenge addressed)						
ANTIGEN DILEMMA (AD)	All trans retinoic acid	Yes	Vitamin A Derived Retinoid	BCMA	MM	36722406
				CD38	MM	36918219
	Azacitidine	Yes	Hypomethylating Agent	CD70	AML	35452603
	Bryostatin-1	Yes	modulate Protein Kinase C	CD22	Leukemias and lymphomas	31110075
				CD22	B-ALL	35222407
	Cisplatin	Yes	Alkylating Agent	CD133	Gastric	33635343
	Crenolanib	Yes	Kinase Inhibitor	FLT3	AML	29472720
	Cyclophosphamide	Yes	Lymphodepleting	NKG2D ligands	Tumor free	26122933
	Decitabine (Dec)	Yes	Hypomethylating Agent	CSPG4	Ovarian	36291817
				CD19	Lymphoblastoma	31372000
	Dec with Chidamide	Yes	with HDAC Inhibitor	nanobodyCD70	AML	36932256
	Gemcitabine	Yes	Antimetabolite	GRP78	Pancreatic	37897831
	Ingenol-3-angelate	Yes	Protein Kinase C Agonist	B7-H3	Osteosarcoma	38561833
	Lenalidomide	Yes	Immunomodulatory Agent	MUC1	MM	35840578
	Lorlatinib	Yes	Broad Kinase Inhibitor	CD19, GD2, ALK	Leukemia, NB	38039964
TUMOR BURDEN (TB)	ABT-737	No	Bcl-2 Inhibitor	CD19	B Cell leukemia	23788110
	Azacitidine	Yes	Hypomethylating Agent	CEA	Colorectal	30075754
	Celecoxib	Yes	COX2 Inhibitor	CD19	B Cell leukemia	29904021
	Dabrafenib	Yes	MAPK Inhibitor	GD2	Melanoma	25415284
	Decitabine	Yes	Hypomethylating Agent	EGFR, CD44v6	Bladder	34868059
	Fluorouracil	Yes	Anti-Metabolite	CEA	Colorectal	30075754
	Indometacin	Yes	NSAID	CD19	B Cell Lymphoma	35882449
	Paclitaxel	Yes	Anti-Microtubule Agent	T4	Epithelial Ovarian	30167862
	Rimiducid	Yes	Dimerizing Agent	IL-1RAP	AML	33414517
	Sodium Butyrate	No	HDAC Inhibitor	CEA	Colorectal	30075754
	THZ1	No	Broad Kinase Inhibitor	EGFR	TNBC	33875483
	Trametinib	Yes	MEK Inhibitor	GD2	Melanoma	25415284
	Vemurafenib	Yes	MAPK Inhibitor	GD2	Melanoma	25415284
	Zanubrutinib	Yes	Src Kinase Inhibitor	CD19	Lymphoblastoma	36254554
IINFILT-RATION (I)	DMXAA	No	STING Agonist	Neu	Breast	33382402
	Docetaxel	Yes	Anti-Neoplastic Agent	HER-2	NSCLC	30744691
	Rapamycin	Yes	mTOR Inhibitor	EpCAM	AML	34233960
IMMUNE MICROENVIRONMENT (IM)	All trans retinoic acid	Yes	Vitamin A Derived Retinoid	FGFR4	Rhabdomyosarcoma	35877472
	BLZ945	No	CSF1R Inhibitor	B7-H3	Glioma	37971169
	Carboplatin	Yes	Alkylating Agent	Lewis Y antigen (LeY)	Prostate	37660083
	Cyclophosphamide (Cy)	Yes	Lymphodepleting	CD19	Raji tumors	21487038
				CD19	Raji tumors	18477047
				CEA	Colon, Breast	33796409
				PSCA	Prostate, Pancreatic	33647456
	Cy with Fludarabine	Yes	with Lymphodepleting	CD19	B Cell Leukemia	25940712
	Cy with Fludarabine	Yes	with Lymphodepleting	B7-H3	Canine Sarcoma	35405743
	Docetaxel	Yes	Anti-Neoplastic Agent	PSMA	Prostate	35962287
	Epacadostat	Yes	IDO1 Inhibitor	FGFR4	Rhabdomyosarcoma	35877472
	Epacadostat	Yes	IDO-1 Inhibitor	MSLN	ESCC	33828565
	L-NAME	No	iNOS Inhibitor	FGFR4	Rhabdomyosarcoma	35877472
	Oxamate	No	LDHA Inhibitor	EGFRvIII	GBM	37770937
	Pexidartinib	Yes	CSF1R Inhibitor	FGFR4	Rhabdomyosarcoma	35877472

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	Drug(s)	FDA Appr.	Drug Description	CAR Target(s)	Cancer Model(s)	PMID
(IM)	PI-3065	No	PI3K inhibitor	ROR1, EGFRvIII	Breast	29760047
	SD-208	No	TGFβ Inhibitor	FGFR4	Rhabdomyosarcoma	36722406
				ROR1	TNBC	32303620
	7DW8-5	No	Immunostimulant	ROR1, EGFRvIII	Breast	29760047
FITNESS (F)	Acalabrutinib	Yes	Src Kinase Inhibitor	CD19	Lymphoblastoma	31899702
	AKT Inhibitor VIII	No	PI3K Inhibitor	CD19	B cell leukemia	29212954
	Carboplatin	Yes	Alkylating Agent	ErbB	Epithelial Ovarian	23898037
				EGFR	TNBC	35813488
	Celecoxib with aspirin	Yes	COX1/2 Inhibitors	CD19	B Cell Lymphoma	34122428
	Dasatinib (Dasa)	Yes	Src Kinase Inhibitor	GD2	B Lymphoid Leukemia	33795428
				GRP78	AML	35102167
				CD19	B Lymphoid Leukemia	30814055
				CD19	Lymphoblastoma	31270272
				CD19	B Lymphoid Leukemia	34289897
	Dasa with Ibrutinib	Yes	Src Kinase Inhibitor	CD7	T cell Leukemia	36086817
	Decitabine	Yes	Hypomethylating Agent	CD19	Lymphoblastoma	33462245
				CD123	Leukemia	32973749
	Dexamethasone (Dex)	Yes	Anti-Inflammatory Synthetic Glucocorticoid	IL13Ra2	GBM	35081104
				IL13Ra2	GBM	29103912
				CD19, CS-1, TAG-72	ALL, MM, Ovarian	38140726
	Dex with methylprednisolone	Yes	Anti-Inflammatory Synthetic Glucocorticoid	CD19, MSLN	Leukemia	38475830
	Docetaxel	Yes	Anti-Neoplastic Agent	PSMA	Prostate	32728611
	Enasidenib	Yes	IDH2 Inhibitor	CD19	Leukemia, Osteosarcoma	38171332
	Ibrutinib	Yes	Src Kinase Inhibitor	CD19	CLL	32683672
				CD19	Lymphoblastoma	32876369
				CD19	MCL	26819453
				CD19	CLL	26813675
				CD19	Lymphoblastoma	31899702
	Idelalisib	Yes	PI3K Inhibitor	CD19	CLL	30737788
	IPI-145, CAL-101, or TGR-1202	No	PI3K Inhibitor	MSLN	Melanoma	32383488
	JQ1	No	BET Bromodomain Inhibitor	EGFR	GBM	34058385
				CD19	CLL	34396987
	Lenalidomide	Yes	Immunomodulatory Agent	CD133, HER2	GBM, Breast	32967454
				CD19, BCMA	Lymphoblastoma	38123696
				NKG2D	Colorectal	37790973
				CD23	CLL	35259043
				CD19	Lymphoblastoma	33408186
				CD19	Lymphoblastic Leukemia	33333026
				BCMA	MM	31395689
				CS1	MM	29061640
	LY294002	No	PI3K Inhibitor	NKG2D	Breast, Lung	35965586
				NKG2D	CML, Pancreatic	30619300
				CD33	AML	29479065
	Metformin	Yes	Antihyperglycemic	CD19	Lymphoma	29662316
	Paclitaxel	Yes	Anti-Microtubule Agent	ICAM-1	Gastric	32995483
	Rapamycin	Yes	mTOR Inhibitor	CD123, HER2,CD33	AML	32384544
				BCMA, CD123 (Natural Killer Cells)	AML	32384544
				IL-1RAP	AML	37173386
				CD19, BCMA	Lymphoma	31039141
				CD19	Lymphoblastoma	30890531
				CD19	Lymphoblastoma	29661681
	Regorafenib	Yes	Broad Kinase Inhibitor	EpCAM (NK Cell)	Colorectal	30410941

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	Drug(s)	FDA Appr.	Drug Description	CAR Target(s)	Cancer Model(s)	PMID
Fitness (F)	Rimiducid	Yes	Dimerizing Agent	SLAMF7	MM	30740516
				CD123, HER2,CD33	AML	30740516
	Ruxolitinib	Yes	Janus Kinase Inhibitor	CD19	Lymphoblastoma	35101664
	SCH-58261	No	A2α Receptor Inhibitor	CD19	Leukemia	29720380
				MSLN	Ovarian	32151275
	Temozolomide	Yes	Alkylating Agent	EGFRvIII	GBM	29872570
	THZ1	No	Broad Kinase Inhibitor	CD19	Lymphoma	33397398
	Trametinib	Yes	MEK Inhibitor	GD2	NB	34382720
MULTI-FUNCTIONAL DRUGS						
AD, F	Azacitidine	Yes	Hypomethylating Agent	CD123	AML	34750374
	Decitabine	Yes	Hypomethylating Agent	NY-ESO-1	Breast, MM	26447882
AD, TB	S63845	No	Mcl-1 Inhibitor	CD19	B-cell Malignancies	33362794
	Venetoclax	Yes	Bcl-2 Inhibitor	CD19	B-cell Malignancies	33362794
				CD19	Multiple Lymphoma Models	35904479
F, I	Cisplatin	Yes	Alkylating Agent	HER2	Lung	38282968
	Lenalidomide	Yes	Immunomodulatory Agent	CD19	B Cell Lymphoma	37219767
				Wilms Tumor 1	CML	34674611
				CD19, CD20	B-cell non-Hodgkin Lymphoma	27141398
				EGFRvIII	GBM	26450624
IM, TB	Sorafenib	Yes	Broad Kinase Inhibitor	GPC3	Hepatocellular	31078430
I, IM	Oxaliplatin (with cyclophosphamide)	Yes	Alkylating Agent	ROR1	Lung	33357452
TB, F	Azacitidine	Yes	Hypomethylating Agent	CD44v6	AML	37180104
	Decitabine	Yes	Hypomethylating Agent	CD44v6	AML	37180104
	Eltanexor	No	XPO-1 Inhibitor	CD19	Lymphoma, AML	34165175
	Ibrutinib	Yes	Src Kinase Inhibitor	CD19	Lymphoblastoma	36254554
	JQ1	No	BET Bromodomain Inhibitor	CD19, CD123	AML	36038554
	Linsitinib	Yes	IGF1R/IR Inhibitor	GD2	Diffuse Midline Glioma	34964902
	Metformin	Yes	Antihyperglycemic mTOR Inhibitor	CEA	Gastric	36827893
	Rapamycin	Yes	mTOR Inhibitor	CD19	B cell Lymphoma	21878902
	Selinexor	Yes	XPO-1 Inhibitor	CD19	Lymphoma, AML	34165175
TB, I	JK184	No	Hedgehog Inhibitor	B7-H3	Breast	36635683
AD, I, IM	Sunitinib	Yes	Broad Kinase Inhibitor	CAIX	Renal	31574023
F, I, TB	Metformin with Rapamycin	Yes	mTOR Inhibitor	EGFRvIII	GBM	38386420
F, I, IM	TY-52156, CAY10444	No	S1P3 Receptor Antagonist	EpCAM	Breast, Colon	37591632

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Figure Legends

Figure 1: Structure of second-generation chimeric antigen receptor. A) CAR molecules on the surface of T cells recognize antigens on the surface of tumor cells and initiate CAR mediated cytotoxicity. **B)** The antigen binding domain is responsible for specifically recognizing the tumor antigen of interest. The blue box represents all antigens listed in **Table 1** but is not an exhaustive list of all targetable antigens. **C)** The hinge region imparts flexibility to the extracellular domain of the CAR molecule to facilitate antigen binding and downstream signal transduction. **D)** The transmembrane domain anchors the CAR molecule into the T cell membrane. **E)** The co-stimulatory domain provides additional support for CAR T cell persistence and viability. **F)** The intracellular signaling domain is ultimately responsible for CAR-mediated cytotoxicity through the initiation of signaling cascades that release cytotoxic granules and cytokines into the tumor microenvironment.

Figure 2: Challenges faced by CAR T cell monotherapy. Successfully implementing CAR T cells for treatment is hindered by both tumor and CAR T cells specific limitations. Large, heterogeneous in makeup, and physically complex solid tumors are often difficult to fully eradicate by singular monotherapies. Coupled with downregulated or heterogenous antigen expression, CAR T cell homing and infiltration is limited. The tumor associated immunosuppressive microenvironment also negatively affects the effector function of CAR T cells. Functionally, CAR T cells effectiveness can be hindered by poor pre- and post- infusion expansion. Premature exhaustion, off target toxicities, and activation induced dysfunction contribute to the incomplete anti-tumor potential of CAR T cells.

Figure 3: Treatment options for CAR T and chemotherapy combination strategies. A) CAR T cells and chemotherapeutics are administered simultaneously; chemotherapeutics may be continuously administered post-infusion. **B)** Tumor cells are (1) primed prior to (2) CAR T cell administration. **C)** CAR T cells are (1) primed prior to (2) infusion. Other timelines may be considered that are a combination of these three basic approaches.

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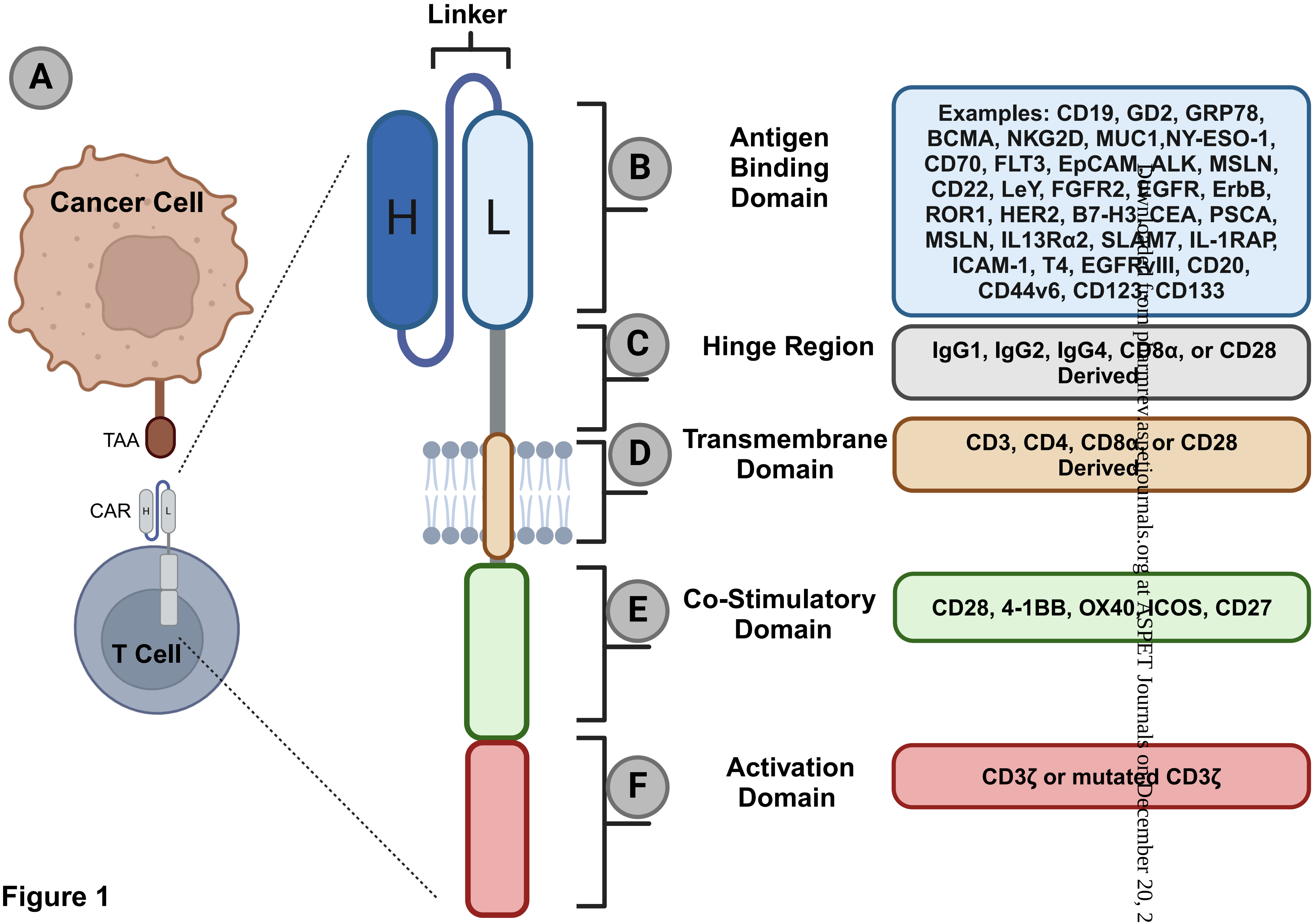

Figure 1

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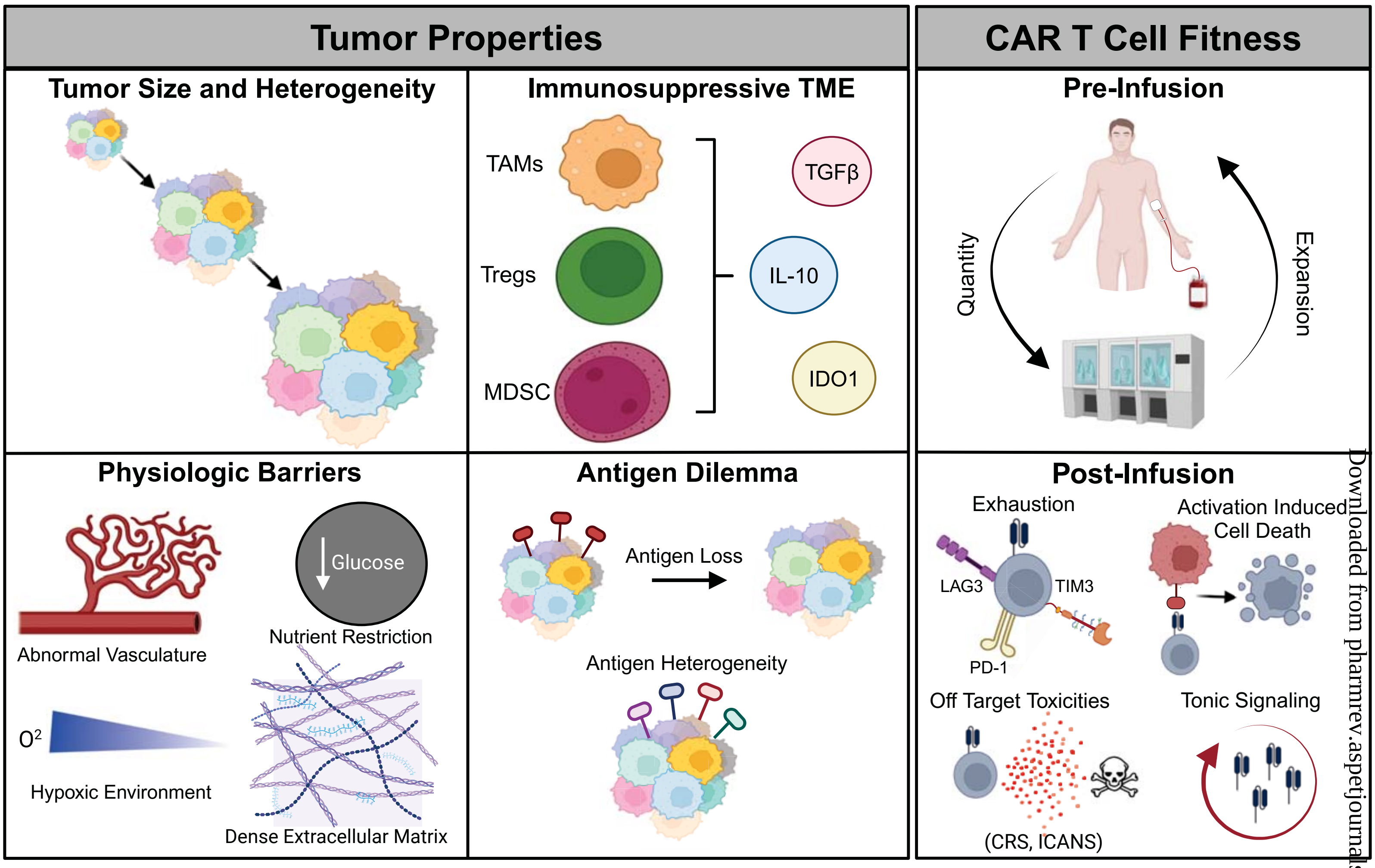

Figure 2

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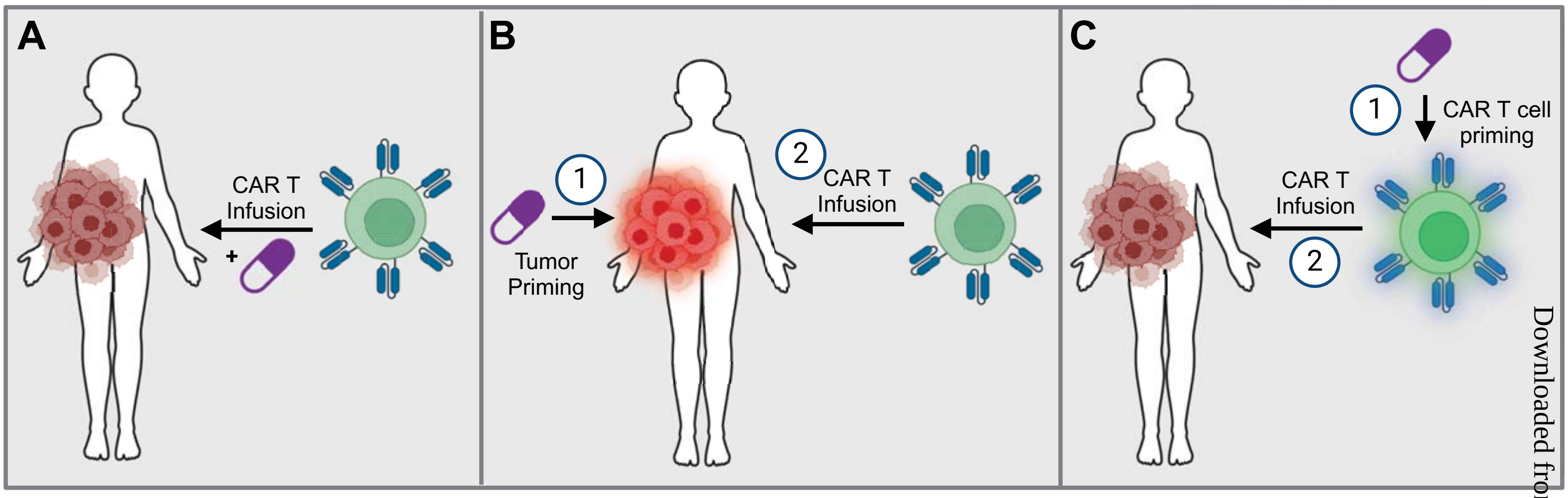

Figure 3

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Therapeutic advantage of combinatorial CAR T cell and chemo-therapies

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Pharmacological Reviwes:
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Supplementary Table 1: Expanded list of preclinical CAR T and chemotherapy studies including relevant mechanisms of action

Drug (s)		FDA Appr. (Y/N)	Drug Description	CAR Target (s)	Tumor Model (s)	CAR Limitation (s) Addressed	Relevant Biological Mechanism(s)	PMID
1	All trans retanoic acid (tretinoin)	Yes	Vitamin A Derived Retinoid	BCMA	Multiple Myeloma	Antigen Dilema	Enhanced antigen expression on primary myeloma cells	36722406
2	All trans retanoic acid (tretinoin)	Yes	Vitamin A Derived Retinoid	CD38	Multiple Myeloma	Antigen Dilema	Upregulated antigen expression on tumor cells which potentiated CAR T cell killing	36918219
3	Azacitidine	Yes	Hypomehylating Agent	CD70	Acute Myeloid Leukemia	Antigen Dilema	Increased antigen expression on tumor cells in a high tumor burden bearing model	35452603
4	Bryostatin-1	Yes	modulate Protein Kinase C	CD22	Leukemias and lymphomas	Antigen Dilema	Upregulated CD22 on different leukemia and lymphoma cells which improved CAR T cell functionality and in vivo persistence	31110075
5	Bryostatin-1	Yes	modulate Protein Kinase C	CD22	B-ALL	Antigen Dilema	Increased cytolysis through enhanced antingen expression on cancer cell lines while sparing normal cells	35222407
6	Cisplatin	Yes	Alkylating Agent	CD133	Gastric Cancer	Antigen Dilema	Pretreament of tumor cells iwith drug mproved tumor cell killing by CAR T cells	33635343
7	Crenolanib	Yes	Kinase Inhibitor	FLT3	Acute Myeloid Leukemia	Antigen Dilema	Upregulated antigen expression leading to increased CAR T cell mediated killing	29472720
8	Cyclophos-phamide	Yes	Lymphodepleting Agent	NKG2D ligands	Off-Target Study Only	Antigen Dilema	Upregulated NKG2D ligands which improved cytotoxicity of CAR T cells	26122933

9	Decitabine	Yes	Hypomehylating Agent	CSPG4	Ovarian Cancer	Antigen Dilema	Mediated a dose dependent increase in CSPG4 on antigen negative cells enhancing recognition by CAR T cells	36291817
10	Decitabine	Yes	Hypomehylating Agent	CD19	Lymphoblastoma	Antigen Dilema	Increased antigen expression on tumor cells improving CAR T cell mediated killing	31372000
11	Decitabine and Chidamide	Yes	Hypomehylating Agent and HDAC Inhibitor	nanobodyCD70	Acute Myeloid Leukemia	Antigen Dilema	Combinatorial treament enhanced the expression of CD70 on AML cells which facilitated greater cytotoxic activity of CAR T cell	36932256
12	Gemcitabine	Yes	Antimetabolite	GRP78	Pancreatic Cancer	Antigen Dilema	Increased surface antigen expression leading to increased tumor killing by CAR T cells	37897831
13	Ingenol-3-angelate	Yes	Protein Kinase C Agonist	B7-H3	Osteosarcoma	Antigen Dilema	Increased surface expression of B7-H3 via PKC activation	38561833
14	Lenalidomide	Yes	Immunomodulatory Agent	MUC1	Multiple Myeloma	Antigen Dilema	Increased antigen expression on tumor cells improving CAR T cell mediated killing	35840578
15	Lorlatinib	Yes	Broad Kinase Inhibitor	CD19, GD2, ALK	Leukemia, Neuroblastoma	Antigen Dilema	Increased antigen expression on low antigen density cells potentiating CAR T cell effector function	38039964
16	ABT-737	No	Bcl-2 Inhibitor	CD19	B Cell Malignancy	Tumor Burden	Increased tumor cell apoptosis	23788110
17	Azacitidine	Yes	Hypomethylating Agent	CEA	Colorectal Cancer	Tumor Burden	Increased antigen expression on tumor cells and improved cytotoxic effect of CAR T cells which may be of resistant to other chemotherapy resistant models	30075754
18	Celecoxib	Yes	COX2 Inhibitor	CD19	B Cell Malignancy	Tumor Burden	Partially reversed CAR T cell resistance of tumor cell lines	29904021

19	Dabrafenib	Yes	MAPK Inhibitor	GD2	Melanoma	Tumor Burden	Used in the clinic to treat melanoma, need to see if can be used in combination but little impact on CAR T cell function individually	25415284
20	Decitabine	Yes	Hypomehylating Agent	EGFR, CD44v6	Bladder cancer	Tumor Burden	Differential regulated the expression of cell survial and apoptosis factors skewing the balance towards more apoptosis sensitivity which enhanced CAR T cell killing	34868059
21	Fluorouracil	Yes	Anti-Metabolite	CEA	Colorectal Cancer	Tumor Burden	Increased antigen expression on tumor cells but negatively influenced the viability of the CAR T cells	30075754
22	Indometacin	Yes	NSAID	CD19	B Cell Lymphoma	Tumor Burden	Sensitized tumor cells to CAR T cells by inducing oxidative stress which led to increased death receptor surface expression	35882449
23	Paclitaxel	Yes	Anti-Microtubule Agent	T4	Epithelial Ovarian Cancer	Tumor Burden	Induced tumor cell apopotosis and promoted cell cycle arrest offerign additive benefit to CAR T cell therapy	30167862
24	Rimiducid	Yes	Dimerizing Agent	IL-1RAP	Acute Myeloid Leukemia	Tumor Burden	Eliminated tumor cells that were resistant to CAR T cell therapy whose resistance was driven by epitope masking	33414517
25	Sodium Butyrate	No	HDAC Inhibitor	CEA	Colorectal Cancer	Tumor Burden	Increased antigen expression on tumor cells and improved cytotoxic effect of CAR T cells which may be of resistant to other chemotherapy resistant models	30075754
26	THZ1	No	Broad Kinase Inhibitor	EGFR	Triple Negative Breast Cancer	Tumor Burden	Overcame tumor acquired CAR T cell resistance thorugh transcriptional reprogramming preventing further resistnace, tumor growth, and metastasis	33875483

27	Trametinib	Yes	MEK Inhibitor	GD2	Melanoma	Tumor Burden	Used in the clinic to treat melanoma, need to see if can be used in combination but little impact on CAR T cell function individually	25415284
28	Vemurafenib	Yes	MAPK Inhibitor	GD2	Melanoma	Tumor Burden	Used in the clinic to treat melanoma, need to see if can be used in combination but compound did impair CAR T cells at plasma relevant concentrations	25415284
29	Zanubrutinib	Yes	Src Kinase Inhibitor	CD19	Lymphoblasto ma	Tumor Burden	Induced dose dependent toxiticty on tumor cells and CAR T cells	36254554
30	DMXAA	No	STING Agonist	Neu	Breast Cancer	Infiltration	Improved infiltrration of raditional Th1 type CAR T cells but more significnalty synergized with Th/Tc17 CAR T cell phenotypes	33382402
31	Docetaxel	Yes	Anti-Neoplastic Agent	HER-2	Non-Small Cell Lung Cancer	Infiltration	Increased the recruitment and infiltrative capacity of CAR T cells through a potential CXCL11 mechanism	30744691
32	Rapamycin	Yes	mTOR Inhbitor	EpCAM	Acute Myeloid Leukemia	Infiltration	Improved the capacity of CAR T cells to infiltrate into the bone marrow by modulating mTOR target CXCR4	34233960
33	All trans retanoic acid (tretinoin)	Yes	Vitamin A Derived Retinoid	FGFR4	Rhabdomyosar coma	Immune Microenvironment	Targets myeloid derived suppressor cells	35877472
34	BLZ945	No	CSF1R Inhibitor	B7-H3	Glioma	Immune Microenvironment	Depleted tumor associated macrophages but was actually detrimental to CAR T cell efficacy	37971169
35	Carboplatin	Yes	Alkylating Agent	Lewis Y antigen (LeY)	Prosate cancer	Immune Microenvironment	Improved the efficacy of CAR T cell treatment but the response was dependent on the changes induced by the tumor microenvironment	37660083

36	Cyclophos-phamide	Yes	Lymphodepleting Agent	CD19	Raji tumors	Immune Microenvironment	Depleted tumor associated Tregs	21487038
37	Cyclophos-phamide	Yes	Lymphodepleting Agent	CD19	Raji tumors	Immune Microenvironment	Contributed to tumor clearance in combination with CAR T cells	18477047
38	Cyclophos-phamide	Yes	Lymphodepleting Agent	CEA	Colon Cancer, Breast Cancer	Immune Microenvironment	Aided in tumor clearance of initially established tumors and helped induce tumor immunity in re-challenged settings	33796409
39	Cyclophos-phamide	Yes	Lymphodepleting Agent	PSCA	Prostate, Pancreatic	Immune Microenvironment	Dampened immunosuppressive microenvironment and supported pro-inflammatory microenvironment	33647456
40	Cyclophos-phamide and Fludarabine	Yes	Lymphodepleting Agent	CD19	B Cell Leukemia	Immune Microenvironment	Downregulated IDO expression	25940712
41	Cyclophos-phamide and Fludarabine	Yes	Lymphodepleting Agent	B7-H3	Canine Sarcoma	Immune Microenvironment	Demonstrated efficacy of CAR T cells in canine sarcoma with cyclophosphamide and fludaribine successfully used as lymphopdepleting agents	35405743
42	Docetaxel	Yes	Anti-Neoplastic Agent	PSMA	Prostate Cancer	Immune Microenvironment	Improved cytotoxic effect of CAR T cells by diminishing the frequency and immunosuppressive capacity of MDSCs	35962287
43	Epacadostat	Yes	IDO1 Inhibitor	FGFR4	Rhabdomyosarcoma	Immune Microenvironment	Promtoted the successsful clearance of RMS tumors using a polypharmacy approach	35877472
44	Epacadostat	Yes	IDO-1 Inhibitor	MSLN	Esophageal Squamous Cell Carcinoma	Immune Microenvironment	Prevented the accumulation of the immunosuppresive metabolite,kynurenine, which enhanced cytotoxic potential	33828565
45	L-NAME	No	iNOS Inhibitor	FGFR4	Rhabdomyosarcoma	Immune Microenvironment	Promtoted the successsful clearance of RMS tumors using a polypharmacy approach	35877472

46	Oxamate	No	Lactate Dehydrogenase A Inhbitor	EGFRvIII	Glioblastoma	Immune Microenvironment	Modulated the immunosuppressive microenvironment by reducing promoter activity of Treg populations and restraining chemokine activity in macrophages	37770937
47	Pexidartinib	Yes	CSF1R Inhibitor	FGFR4	Rhabdomyosarcoma	Immune Microenvironment	targets M2 like TAMs	35877472
48	PI-3065	No	PI3K Inhibitor	ROR1, EGFRvIII	Breast	Immune Microenvironment	Nanoparticle loaded sheets improved CAR T cell effector function through remodeling of the immune microenvironment	29760047
49	SD-208	No	TGFβ Inhibitor	FGFR4	Rhabdomyosarcoma	Immune Microenvironment	Protected CAR T cells from inhibitory effect of TGF-β and reduced PD-1 expression within a polypharmacy approach	36722406
50	SD-208	No	TGFβ Inhibitor	ROR1	Triple-Negative Breast Cancer	Immune Microenvironment	Protected CAR T cells from inhibitory effect of TGF-β and reduced PD-1 expression within a polypharmacy approach	32303620
51	7DW8-5	No	Immunostimulatory Glycolipid	ROR1, EGFRvIII	Breast	Immune Microenvironment	Nanoparticle loaded sheets improved CAR T cell effector function through remodeling of the immune microenvironment	29760047
52	Acalabrutinib	Yes	Src Kinase Inhibitor	CD19	Lymphoblastoma	Fitness	Improved in vitro persistence and in vivo tumor clearance,	31899702
53	AKT Inhbitor VIII	No	PI3K Inhibitor	CD19	B cell leukemia	Fitness	Minimized differentiation of CAR T cells through induced regulation of the FOXO1 transcription factor	29212954
54	Carboplatin	Yes	Alkylating Agent	ErbB	Epithelial ovarian cancer	Fitness	Tumor pre-treatment with noncytotoxic doses of carboplatin sensitized EOC tumors to CAR T cells resulting in better disease regression while using lower doses of CAR T cells	23898037

55	Carboplatin	Yes	Alkylating Agent	EGFR	Breast cancer (TNBC; MDA-MDB-468 cells)	Fitness	Improved CAR T cell cytotoxic activity in vitro	35813488
56	Celecoxib and aspirin	Yes	COX1/2 Inhibitors	CD19	B Cell Lymphoma	Fitness	COX inhibition induced tumor cell apoptosis but impaired quality of CAR T cells	34122428
57	Dasatinib	Yes	Src Kinase Inhibitor	GD2	B Lymphoid Leukemia	Fitness	Promoted memory phenotype, decreased exhaustion markers, and increased effector cytokine production	33795428
58	Dasatinib	Yes	Src Kinase Inhibitor	GRP78	Acute Myeloid Leukemia	Fitness	Prevented CAR T cell antigen mediated differentation which improved cytotoxicity	35102167
59	Dasatinib	Yes	Src Kinase Inhibitor	CD19	B Lymphoid Leukemia	Fitness	Served as a safety switch for CAR T cell by limiting cytotoxicity, cytokine secretion, and proliferation	30814055
60	Dasatinib	Yes	Src Kinase Inhibitor	CD19	Lymphoblastoma	Fitness	Paused CAR T cell intracellullar signaling transduction by interefering with upstream kinase activity	31270272
61	Dasatinib	Yes	Src Kinase Inhibitor	CD19	B Lymphoid Leukemia	Fitness	Decreased exhaustion, differentiation and apoptosi related gene signatures	34289897
62	Dasatinib and Ibrutinib	Yes	Src Kinase Inhibitor	CD7	T cell Leukemia	Fitness	Minimized tonic signaling induced fratricide on the CAR T cell and improved persistance and tumor control	36086817
63	Decitabine	Yes	Hypomehylating Agent	CD19	Lymphoblastoma	Fitness	Enhanced memory phenotype, proliferation, and cytokine production of CAR T cells while also mitigating exhaustion	33462245

64	Decitabine	Yes	Hypomehylating Agent	CD123	Leukemia	Fitness	Epigenetically reprogammed CAR T cells which decreased methyltransferase expression leading to decreased exhaustion markers and ehnanced memory phenotype	32973749
65	Dexamethasone	Yes	Anti-Inflammatory Synthetic Glucocorticoid	IL13Ra2	Glioblastoma	Fitness	Identified threshold that which dexamethasone is harmful for CAR T cells	35081104
66	Dexamethasone	Yes	Anti-Inflammatory Synthetic Glucocorticoid	IL13Ra2	Glioblastoma	Fitness	Dex is commonly used in GBM tretament, low doses do not impact CAR T cell functionality - but do not necessarially help either	29103912
67	Dexamethasone	Yes	Anti-Inflammatory Synthetic Glucocorticoid	CD19, CS-1, TAG-72	ALL, Multiple Myeloma, Ovarian Cancer	Fitness	Dex increases IL-7 receptor expression on CAR T cells, further combination with IL-7 enhanced anti-tumor activity	38140726
68	Dexamethasone and methylpred-nisone	Yes	Anti-Inflammatory Synthetic Glucocorticoid	CD19, MSLN	Leukemia	Fitness	Exposure to GCs impairs CAR T cell activity but is reversed when GCs are removed, Important when GCs are used to control CRS but cytotoxicity is still desired	38475830
69	Docetaxel	Yes	Anti-Neoplastic Agent	PSMA	Prostate Cancer	Fitness	Combinatorial treatment proved to have additive effect at killing antigen positive tumor cells	32728611
70	Enasidenib	Yes	IDH2 Inhibitor	CD19	Leukemia, Osteosarcoma	Fitness	Enhances persistence, expansion, and memory CAR T cell formation through enhanced glycolytic activity and reduced reacitve oxygen species formation	38171332
71	Ibrutinib	Yes	Src Kinase Inhibitor	CD19	Chronic Lymphocytic Leukemia	Fitness	Increased CAR T cell viability and expansion while decreasing differentiation and exhaustion markers and imporving cytokine release	32683672

72	Ibrutinib	Yes	Src Kinase Inhibitor	CD19	Lymphoblasto ma	Fitness	Decreased exhaustion marker and displayed better combined tumor control when added to CAR T cells	32876369
73	Ibrutinib	Yes	Src Kinase Inhibitor	CD19	Mantle Cell Lymphoma	Fitness	Enhanced CAR T cell killing and improved long term remission of mice and exhaustion markers in vivo	26819453
74	Ibrutinib	Yes	Src Kinase Inhibitor	CD19	Chronic Lymphocytic Leukemia	Fitness	Improves CAR T cell engraftment supporting greater tumor clearence	26813675
75	Ibrutinib	Yes	Src Kinase Inhibitor	CD19	Lymphoblasto ma	Fitness	Improved in vitro persistence, in vivo tumor clearance,and promoted a memory phenotype.	31899702
76	Idelaslisib	Yes	PI3K Inhibitor	CD19	Chronic Lymphocytic Leukemia	Fitness	Enriched the naive T CAR T cell fraction, increased lymph node homing markers, and decreased exhaustion marker expression	30737788
77	IPI-145,CAL-101, or TGR-1202	No	PI3K Inhibitor	MSLN	Melanoma	Fitness	Dual blockade of the gamma and delta subunits of PI3K impaired cell function but individual use with either inhibitor improved anti-tumor function	32383488
78	JQ1	No	BET Bromodomain Inhibitor	EGFR	Glioblastoma	Fitness	Suppressed the transcriptional expression of CAR T cell induced immunosuppressive genes supporting cytotoxic argeting of tumor cells	34058385
79	JQ1	No	BET Bromodomain Inhibitor	CD19	Chronic Lymphocytic Leukemia	Fitness	Reinvigorated exhausted CAR T cells through increased CAR expansion, proliferation, and metabolic fitness	34396987
80	Lenalidomide	Yes	Immunomodulatory Agent	CD133, HER2	Glioblastoma, Breast Cancer	Fitness	Increased CAR T effector function (prolerfation, cytokine secretion,and killing) through drug regulated degradation of T cell essential transcription factors	32967454

81	Lenalidomide	Yes	Immunomodulatory Agent	CD19, BCMA	Lymphoblastoma	Fitness	Drug regulated degradable IL-7 system that enhanced CAR T cell expansion, memory status, and persistence and antitumor activity of CAR T cells	38123696
82	Lenalidomide	Yes	Immunomodulatory Agent	NKG2D	Colorectal Cancer	Fitness	Improved in vitro cytotoxicity and effector cytokine production	37790973
83	Lenalidomide	Yes	Immunomodulatory Agent	CD23	Chronic Lymphocytic Leukemia	Fitness	Improved CAR T cell migration and preserved the functionality of CAR T cell immune synapse improving effector function	35259043
84	Lenalidomide	Yes	Immunomodulatory Agent	CD19	Lymphoblastoma	Fitness	Served as an "ON" and "OFF"supporting the activation or degradation of CAR T cells which improved antitumor activity while limiting inflammatory cytokine release	33408186
85	Lenalidomide	Yes	Immunomodulatory Agent	CD19	Lympoblastic Leukemia	Fitness	Controlled effector function of degron tagged CAR T cells to better regulate activity and minimize off-target toxicitiies	33333026
86	Lenalidomide	Yes	Immunomodulatory Agent	BCMA	Multiple Myeloma	Fitness	Enhanced cytokine production, cytolytic activity, activation, and decreased exhaustion markers improving in vivo tumor clearance	31395689
87	Lenalidomide	Yes	Immunomodulatory Agent	CS1	Multiple Myeloma	Fitness	Supported increased cytotoxicity and persistence in vivo with improved memory phenotype, Th1 cytokine production,and immune synapse formation	29061640
88	LY294002	No	PI3K Inhibitor	NKG2D	Breast Cancer, Lung Cancer	Fitness	Supported CAR T cell survival and prevented exhaustion by upregulating anti-apoptotic proteins	35965586
89	LY294002	No	PI3K Inhibitor	NKG2D	Chronic Myeloid Leukemia, Pancreatic Cancer	Fitness	Decreased antigen expression on activated CAR T cells diminishing fratricididic cell death	30619300

90	LY294002	No	PI3K Inhibitor	CD33	Acute Myeloid Leukemia	Fitness	Improved persisitence by mediating a less differentiated state and decreasing tonic CAR T cell signaling	29479065
91	Metformin	Yes	Antihyperglycemic	CD19	Lymphoma	Fitness	Suppressed the AMPK pathway which negatively influenced proliferation and cytotoxicity of CAR T cells	29662316
92	Paclitaxel	Yes	Anti-Microtubule Agent	ICAM-1	Gastric Cancer	Fitness	Enhanced anti-tumor activity of CAR T cells in vivo	32995483
93	Rapamycin	Yes	mTOR Inhibitor	CD123,HER2, CD33	Acute Myeloid Leukemia	Fitness	Used as a pro-apoptotic compound that allowed for in vivo clearance control of CAR cells to better mitigate off target toxicities	32384544
94	Rapamycin	Yes	mTOR Inhbitor	BCMA,CD123 (Natural Killer Cells)	Acute Myeloid Leukemia	Fitness	Used as a safety switch to regulate the elimination of expanded effector cells	32384544
95	Rapamycin	Yes	mTOR Inhbitor	IL-1RAP	Acute Myeloid Leukemia	Fitness	Used as a safety switch to regulate the elimination of expanded effector cells	37173386
96	Rapamycin	Yes	mTOR Inhbitor	CD19,BCMA	Lymphoma	Fitness	Allowed for selective activation of CAR T cells only in the presence of both the antigen and the dimerizing compound	31039141
97	Rapamycin	Yes	mTOR Inhbitor	CD19	Lymphoblastoma	Fitness	Promoted a less differentiated, stem cell like memory phenotype	30890531
98	Rapamycin	Yes	mTOR Inhbitor	CD19	Lymphoblastoma	Fitness	Utilized rapamycin as a caspase regulating compound to initiate CAR T cell suicide and minimize toxicity	29661681
99	Regorafenib	Yes	Broad Kinase Inhibitor	EpCAM (Natural Killer Cell)	Colorectal Cancer	Fitness	Supported greated in vivo tumor control through enhanced persistence	30410941
100	Rimiducid	Yes	Dimerizing Agent	SLAMF7	Multiple Myeloma	Fitness	Performed as a suicide switch on CAR T cells to better control off target toxicities to antigen containing leukocytes	30740516

101	Rimiducid	Yes	Dimerizing Agent	CD123,HER2, CD33	Acute Myeloid Leukemia	Fitness	Used as a pro-stimulatory compound that enhanced CAR T cell proliferation, cytokine secretion, and cytotoxicity	30740516
102	Ruxolitinib	Yes	Janus Kinase Inhibitor	CD19	Lymphoblastoma	Fitness	Inhibited proliferation without damaging viability or effector function of the CAR T cells which helped mitigate cytokine release syndrome related toxicities	35101664
103	SCH-58261	No	A2α Receptor Inhibitor	CD19	Leukemia	Fitness	CAR T cells were loaded with vessicles carrying SCH supporting higher CAR T cell engraftment and enhanced functionality	29720380
104	SCH-58261	No	A2α Receptor Inhibitor	MSLN	Ovarian Adenocarcinoma	Fitness	Adenosine signling was exogenously activated and co-incubation with SCH reversed loss of CAR T cell effector function and cytokine secretion	32151275
105	Temozolomide	Yes	Alkylating Agent	EGFRvIII	Glioblastoma	Fitness	Served as a lymphodepleting agent prior to CAR delivery which improved the abundance and persistence of cells	29872570
106	THZ1	No	Broad Kinase Inhibitor	CD19	Lymphoma	Fitness	Protecte against cytokine release syndrome assocaited toxicities by diminishing polymerase medaited transciriptional activity	33397398
107	Trametinib	Yes	MEK Inhibitor	GD2	Neuroblastoma	Fitness	Enhanced in vitro tumor cell killing while sparing exhaustion and promoting expansion	34382720
108	Azacitidine	Yes	Hypomehylating Agent	CD123	Acute Myeloid Leukemia	Antigen Dilemma, Fitness	Upregulated antigen expression and induced clonal outgrowth of exhuastion marker negative, CAR positive T cells that had increased killing potency	34750374

109	Decitabine	Yes	Hypomehylating Agent	NY-ESO-1	Breast Cancer, Multiple Myeloma	Antigen Dilemma, Fitness	Upregulated antigen expression on tumor cells leading to increased killing which also correlated with higher interferon gamma production	26447882
110	S63845	No	Mcl-1 Inhibitor	CD19	B-cell Malignancies	Antigen Dilemma/ Tumor Burden	Increased CD19 expression and induced tumor cell apoptosis	33362794
111	Venetoclax	yes	Bcl-2 Inhibitor	CD19	B-cell Malignancies	Antigen Dilemma/ Tumor Burden	Increased CD19 expression and induced tumor cell apoptosis where one out of three delivery methods tested was able to prevent CAR T cell damage	33362794
112	Venetoclax	yes	Bcl-2 Inhibitor	CD19	Multiple Lymphoma Models	Antigen Dilemma/ Tumor Burden	Overexpressed Bcl2(F104L) to protect CAR T cellf from venetoclax toxicities	35904479
113	Cisplatin	Yes	Alkylating Agent	HER2	Lung Cancer	Fitness, Infiltration	Cisplatin-loaded CAR T cells (via membrane coated polymeric nanoparticles) resulted in better anti-tumor response and homing.	38282968
114	Lenalidomide	Yes	Immunomodulatory Agent	CD19	B Cell Lymphoma	Fitness, Infiltration	Polarized CD8+ cells to less differentiated state, increased expansion, suppressed VEGF production supporting infiltration, and minimized exhaustion	37219767
115	Lenalidomide	Yes	Immunomodulatory Agent	Wilms Tumor 1	Chronic Myelogenous Leukemia	Fitness, Infiltration	Increased infiltration of both CD3/8+ T cells and improves anti-tumor activity	34674611
116	Lenalidomide	Yes	Immunomodulatory Agent	CD19,CD20	B-cell non-Hodgkin Lymphoma	Fitness, Infiltration	Increased interferon gamma production and infiltration of CAR positive cells which supported greater anti-tumor activity	27141398
117	Lenalidomide	Yes	Immunomodulatory Agent	EGFRvIII	Glioblastoma	Fitness, Infiltration	Improved interferon gamma production, migration of CAR T cells to tumor site, and enhanced immune synapse formation improving tumor control	26450624

118	Sorafenib	Yes	Broad Kinase Inhibitor	GPC3	Hepatocellular Cancer	Immune Microenvironment, Tumor Burden	Promtoed IL-12 secretion in tumor associated macrophages and induced tumor cell apoptosis	31078430
119	Oxaliplatin (with cyclophos-phamide)	Yes	Alkylating Agent	ROR1	Lung Cancer	Infiltration, Immune Microenvironment	Improves CAR-T cell-mediated tumor control and survival when combined with cyclophosphamide by activating tumor macrophages to express T-cell-recruiting chemokines, resulting in improved CAR-T cell infiltration, remodeling of the tumor microenvironment, and increased tumor sensitivity to anti-PD-L1	33357452
120	Azacitidine	Yes	Hypomehylating Agent	CD44v6	Acute Myeloid Leukemia	Tumor Burden, Fitness	Increased the activation, persistence, and promoted a memory phenotype of CAR T cells while also inducing tumor celll apoptosis	37180104
121	Decitabine	Yes	Hypomehylating Agent	CD44v6	Acute Myeloid Leukemia	Tumor Burden, Fitness	Increased the activation, persistence, and promoted a memory phenotype of CAR T cells while also inducing tumor celll apoptosis (more profound than azacitidine)	37180104
122	Eltanexor	No	XPO-1 Inhibitor	CD19	Lymphoma, Acute Myeloid Leukemia	Tumor Burden, Fitness	Pretreament of tumor cells with inhibitor sensistized them to CAR T cell therapy by decreasing tumor cell viability and mitigating exhaustion	34165175
123	Ibrutinib	Yes	Src Kinase Inhibitor	CD19	Lymphoblasto ma	Tumor Burden, Fitness	Induced dose dependent toxiticty on tumor cells and CAR T cells but decreased exhaustion markers and had better anti-tumor effect	36254554

124	JQ1	No	BET Bromodomain Inhibitor	CD19,CD123	Acute Myeloid Leukemia	Tumor Burden, Fitness	Decreased immunosuppressive ligand on tumor cells and their respective receptors on CAR T cells supporting anti-tumor activity	36038554
125	Linsitinib	Yes	IGF1R/IR Inhibitor	GD2	Diffuse Midline Glioma	Tumor Burden, Fitness	Induced tumor cell apoptosis while not affecting CAR T cell viability and increased memory phenotype expression as well as decreased exhaustion markers	34964902
126	Metformin	Yes	Antihyperglycemic	CEA	Gastric Cancer	Tumor Burden, Fitness	Blocked tumor specific aerobic glycolysis while upregulating CAR T cell oxidative phosphorylation and energy metabolism	36827893
127	Rapamycin	Yes	mTOR Inhbitor	CD19	B cell Lymphoma	Tumor Buden Fitness,	Inhibited tumor specific immune evasion and anti-apoptic mechanisms while working in tandem withengineered rapamycin resistant CAR T cells	21878902
128	Selinexor	Yes	XPO-1 Inhibitor	CD19	Lymphoma, Acute Myeloid Leukemia	Tumor Burden, Fitness	Pretreament of tumor cells with inhibitor sensistized them to CAR T cell therapy by decreasing tumor cell viability and mitigating exhaustion	34165175
129	JK184	No	Hedghog Inhbibitor	B7-H3	Breast Cancer	Tumor Burden, Infiltration	Promoted tumor cell apoptosis and CAR T cell traficking and infiltration to the tumor site which corresponded to increased inflammatory cytokine production	36635683
130	Sunitinib	Yes	Broad Kinase Inhibitor	CAIX	Renal Cancer	Antigen Dilemma, Infiltration, Immune Microenvironment	Upregulated antigen expression on tumor cells, promoted CAR T cell infiltration, and reduced the abundance and frequency of myeloid derived suppressor cells	31574023

131	Metformin and Rapamcyin	Yes	AMPK Activator, mTOR Inhibitor	EGFRvIII	Glioblastoma	FItness, Immune Microenvironment, Tumor Burden	Pretreated CAR T cells had better persistence and anti-tumor activity under impairing hypoxic conditions along with fewer MDSCs	38386420
132	TY-52156	No	S1P3 Receptor Antagonist	EpCAM	Breast Cancer, Colon Cancer	Fitness, Infiltration, Immune Microenvironment	Improved infiltratiion and effector function by remodeling the TME to promote pro-inflammatory macrophage polarization and recruitment	37591632
