 doi:10.20944/preprints202010.0652.v1

Review

Strategies to improve chimeric antigen receptors therapies for neuroblastoma

Piamsiri Sawaisorn,[1,#] Korakot Atjanasuppat,[1,#] Usanarat Anurathapan,[1] Somchai Chutipongtanate,[2,3,*] Suradej Hongeng[1,*]

[1] Division of Hematology and Oncology, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400 Thailand; piamsiri.saw@mahidol.ac.th (P.S.); korakot.atj@mahidol.ac.th (K.A.); usanarat.anu@mahidol.ac.th (U.A.)

[2] Pediatric Translational Research Unit, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400 Thailand

[3] Department of Clinical Epidemiology and Biostatistics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400 Thailand

# Shared equal contribution

* Shared co-corresponding authors: schuti.rama@gmail.com (S.C.), suradej.hon@mahidol.ac.th (S.H)

Abstract: Chimeric antigen receptors (CARs) is one of the curative immunotherapeutic approaches that exploit the antigen specificity and cytotoxicity function of potent immune cells against cancers. Neuroblastoma, the most common extracranial pediatric solid tumors with diverse comportment, could be a promising candidate for using CARs therapies. Several methods harness CARs modified cells in neuroblastoma to increase therapeutic efficiency, albeit the assessment has still been less successful. Regarding the improvement of CARs, various trials have been launched to overcome insufficient capacity. However, the reason behind the inadequate response against neuroblastoma of CARs modified cells are still not well understood. It is essential to update the present reveal of comprehension of CARs to improve the efficiency of CARs therapies. This review summarizes the crucial features of CARs and its design for neuroblastoma, discusses challenges that impact the outcomes of the immunotherapeutic competence, and focuses on devising strategies currently investigated to improve the efficacy of CARs for neuroblastoma immunotherapy.

Keywords: CAR T cells; immunotherapy; pediatric neuroblastoma; strategy

1. Introduction

Neuroblastoma, an extracranial solid tumor that initiates from the sympathetic nervous system's neuroendocrine tissue, is one of the most common causes of death in pediatric cancers [1,2]. It is often diagnosed during the perinatal period, which accounts for 8% in patients under 15 years. This childhood neoplasm appears each year in more than 600 cases in the United States and 200 in Japan [3-5]. According to clinical presentation, neuroblastoma is an extremely variant characteristic tumor. It ranges from an adrenal mass tumor that regresses without treatment to a metastatic tumor that causes critical illness [6]. At present, while intensive therapies can be beneficial for patients with localized disease, these therapies have frequently not been useful against patients with high-risk disease (approximately 40% of cases associated with the extent of metastases and genetic factors) nor patients with relapsed [7,8]. Hence, novel therapies based on immunotherapy are subsequently developed to improve survival for high-risk patients.

One such approach is treatment with anti-GD2 monoclonal antibody, which has already been assessed in the nodal phase III clinical trial. The use of this antibody-based therapy was compiled into a therapeutic protocol for high-risk neuroblastoma patients and disclosed the promising results [9,10]. This effectiveness has led other immunotherapeutic approaches, even though their integration into conventional multimodality therapies requires further investigation.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 October 2020 doi:10.20944/preprints202010.0652.v1

After discovering that GD2, a disialoganglioside highly expressed in most neuroblastomas, is also targeted by T cells, cellular immunotherapies including genetic engineering of T lymphocytes to express anti-GD2 chimeric antigen receptor (CAR) have emerged and now being studied. With the combination of antigen specificity and cytolytic capacity, anti-GD2 CAR T cells have demonstrated safety and antitumor efficacy in relapsed neuroblastoma patients [11,12]. Various preclinical studies have improved antitumor effects, proliferation, and cytokine release of CAR T cells, and some approaches have reached clinical trials (**Figure 1**). Currently, anti-GD2 CAR T cells approach might represent the potential therapeutic for pediatric neuroblastoma.

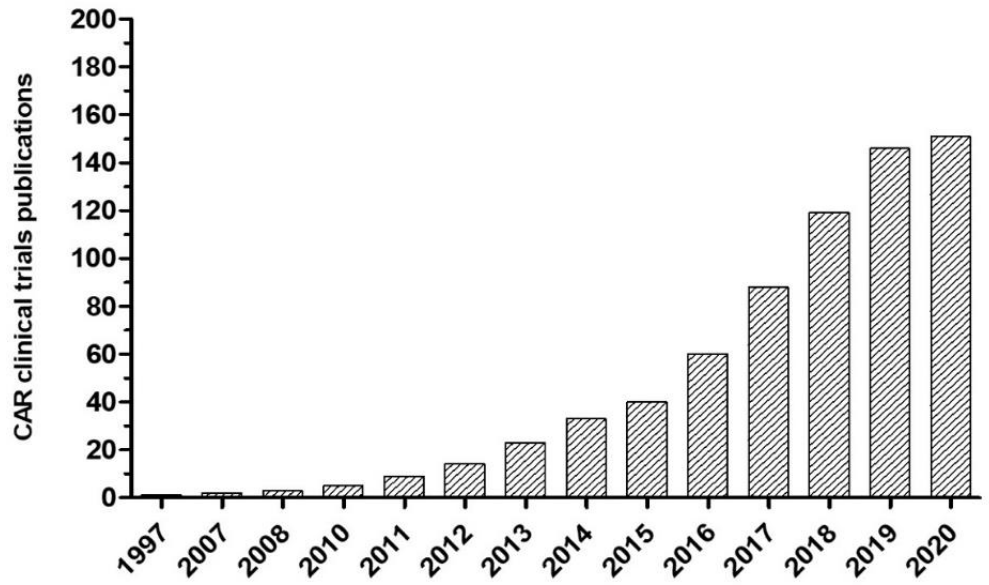

Figure 1. Cumulative publications of CAR immunotherapy in clinical trials.

However, unlike the success of using CAR T cells in hematological malignancies [13-15], the efficacy of CAR T cells in neuroblastomas has been limited by several factors, including tumor microenvironment, T cells exhaustion, and T cells persistence and potency, which may lead to therapeutic resistance [16-18]. Therefore, CAR T cell improvement as a feasible alternative to conventional therapies for patients with neuroblastoma is still a significant challenge to immunotherapy achievement.

In this review, we provide an updated summary of preclinical and clinical experience of CAR-based neuroblastoma therapies and discuss the improvements of CAR in different ways that could overcome clinical problems of applying this approach for treating neuroblastoma. Defining these strategies would suggest an attractive route of improving the potency of CAR immunotherapy.

2. CARs in neuroblastoma

CARs have been developed to fulfill the applicability of adoptive cellular immunotherapy for neuroblastoma in a major histocompatibility complex (MHC)-unrestricted manner of effector T cells. Effector immune cells, commonly T lymphocytes, have been genetically engineered to express an extracellular antigen-binding domain that is mostly single-chain variable fragment (scFv) joined with a transmembrane domain and intracellular signaling domain. The 1st generation CARs were designed to have a single CD3- ζ intracellular signaling domain. The 2nd and 3rd generation CARs products were improved by adding one or two costimulatory endodomains to the CD3- ζ motif to achieve the optimal activation and survival of CAR cells. Current intracellular endodomain based on the costimulatory receptors include CD27, CD28, 41BB, ICOS, and OX40 [17,19]. Each of the CAR design components reflects the variations of therapeutics achievement, and novel CAR engineering has been developed for decades to broaden CARs therapeutics in solid tumors like neuroblastoma.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 October 2020 doi:10.20944/preprints202010.0652.v1

2.1 Summary of CARs experience

Several CAR approaches in neuroblastoma have been developed according to discovered putative cancer antigens. There are some novel target antigens for CAR T cell therapy in neuroblastoma that have been investigated in the preclinical phase (**Figure 2**).

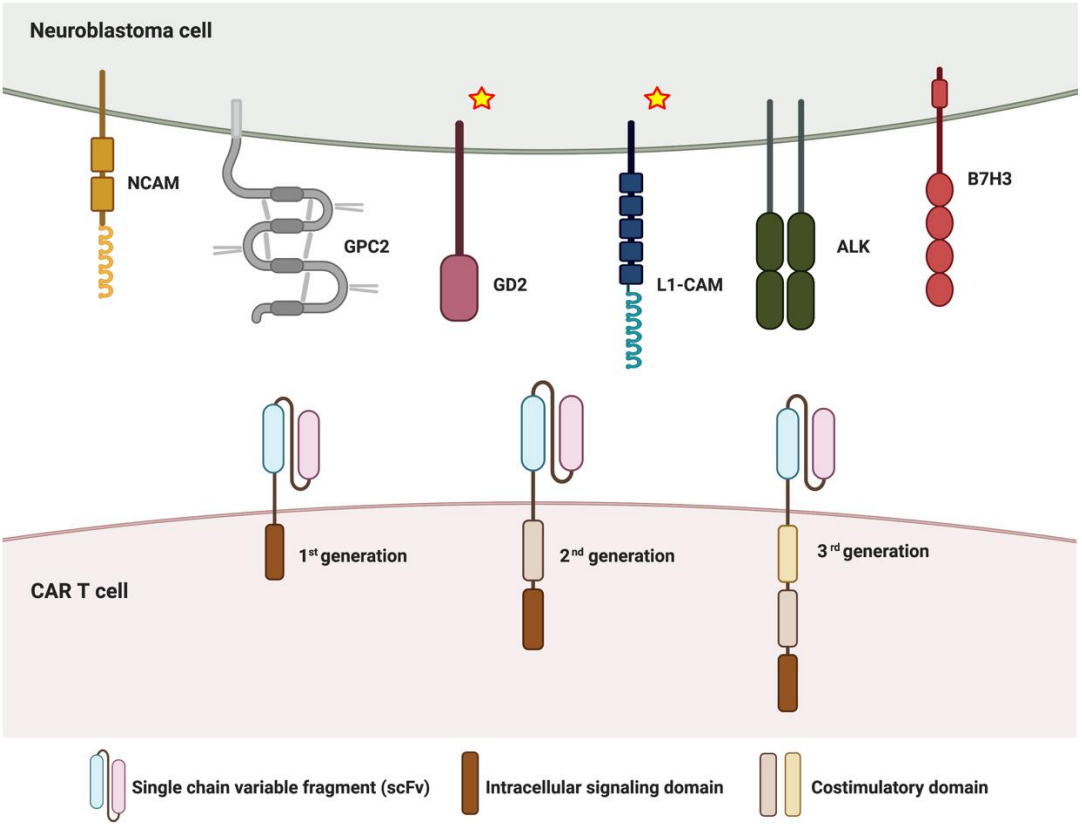

Figure 2. Target antigens conducted on the safety and efficacy of CAR therapy for neuroblastoma. Six surface antigens of neuroblastoma, including L1-CAM, GPC2, NCAM, GD2, ALK, and B7H3, are under development and investigation. L1-CAM and GD2 are only two target antigens currently in completed clinical trials for neuroblastoma (labeled with star).

Anaplastic lymphoma kinase (ALK), an oncogene expressed on neuroblastoma cells, is associated with familial neuroblastoma cases [20,21]. Anti-ALK CAR has demonstrated its effectiveness against this neuroblastoma subtype *in vitro* and *in vivo* [22,23]. This line of research also suggested that antigen density must be concerned to achieve CAR T cell potential. Another tyrosine kinase receptor that may be rendered an ideal target for CAR therapies is glypican 2 (GPC2). The high expression of GPC2 on neuroblastoma cell surface brought promising clearance of disseminated neuroblastoma in the mouse model by anti-GPC2 CAR T cells [24]. B7H3 (CD276), a checkpoint molecule expressed on neuroblastoma, is another candidate for CAR therapies of neuroblastoma [25,26]. This attractive target brought useful immunotherapeutic strategies, including monoclonal antibodies and CAR targeting B7H3. Recently, the efficacy of anti-B7H3 CAR has been being demonstrated *in vivo* [27,28]. Also, many target antigens that are specific to neuroblastoma cells have been more characterized. Such antigens, including NCAM (CD56), NY-ESO1, and PRAME, were investigated both *in vitro* and *in vivo* for safety and efficacy, which gained attention for further development as CAR features [29-32].

To date, only CAR T cells targeting L1-CAM (CD171) and GD2 have been reached the early phase of clinical trials [33] (**Table 1**). L1-CAM, an adhesion molecule in the immunoglobulin superfamily, is another suitable target in neuroblastoma [34]. Because of the specificity of CE7, the monoclonal antibody that can bind to the L1-CAM epitope, the anti-L1-CAM CAR with the scFv from CE7 was generated. This 1st generation anti-L1-CAM CAR efficacy and safety was investigated in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 October 2020 doi:10.20944/preprints202010.0652.v1

patients with relapsed/refractory neuroblastoma in phase 1 clinical trial [12]. To augment the persistence of anti-L1-CAM CAR, 2nd generation CAR was generated using a 41BB costimulation domain following 3rd generation CAR, including CD28 costimulation addition, which is currently being investigated in phase 1 clinical trials [35,36]. Until now, the most critical target antigen in neuroblastoma is GD2, a disialoganglioside highly expressed on neuroblastoma tissue [37]. Owing to the presence of this antigen during chemotherapy and the success of anti-GD2 monoclonal antibody therapy, this antigen has been the most studied targeted for CAR T cell therapy in neuroblastoma [38]. Many approaches of 1st generation of anti-GD2 CAR have been reports, including anti-GD2 CAR containing single-chain variable fragment (scFv) derived from 14g2a monoclonal antibody or Epstein-Barr virus-specific cytotoxic T cells transduced CAR (so-called GD2 CAR-CTL) with the fact that the prolonged persistence *in vivo* was associated with costimulation domain of CAR [39-42]. Anti-GD2 CAR constructs are now considered on costimulatory endodomains. The 2nd and 3rd generations of CAR were then generated for *in vitro* and *in vivo* assessments on CAR T cell survival [43,44]. The 3rd generation anti-GD2 CAR, containing an inducible caspase 9 (iC9) safety switch, has been tested in clinical trials for its safety (clinicaltrials.gov identifier NCT01953900 and NCT01822652). Various clinical trials based on CAR therapy are underway to augment the reliable therapeutic outcomes. However, improving the efficacy and persistence of CAR is still a significant issue.

Table 1. An outline of clinical trials for CAR immunotherapy in neuroblastoma.

Clinicaltrials.gov identifier	Type of study	Target/ScFv/ signaling domain	Key results	Reference
N/A	Phase I N=6	L1-CAM/CE7R/ CD3ζ	Partial response in one patient	[12]
NCT02761915	Phase I N=27	GD2/KM8138/ CD28.CD3ζ	On target activity in bone and bone marrow were detected	[42]
NCT01822652	Phase I N=11	GD2/14g2a/ CD28.OX40.CD3ζ	The modest early antitumor responses were detected	[44]
NCT00085930	Phase I N=19	GD2/14g2a/ CD3ζ	Complete response in one patient	[18,45]
NCT03635632	Phase I N=94	GD2/14g2a/ CD28.OX40.CD3ζ	N/A	Unpublished
NCT01953900	Phase I N=26	GD2/14g2a/ CD28.OX40.CD3ζ	N/A	Unpublished
NCT02107963	Phase I N=15	GD2/14g2a/ CD28.OX40.CD3ζ	N/A	Unpublished
NCT03373097	Phase I/II N=42	GD2/14g2a/ CD28.4-1BB.CD3ζ	N/A	Unpublished

2.2 Obstacles of using CARs in neuroblastoma

2.2.1 CAR T cells persistence and exhaustion

Restrictive CAR T cell persistence has occurred as a major problem in neuroblastoma. Evidence from 1st generation of CAR studies *in vivo* and clinical trials suggested that the limited persistence of CAR T cells from low activation and proliferation of cells also affected the antitumor efficacy [18,43,45,46]. One clinical study demonstrated that the infused, 1st generation, anti-L1-CAM CAR cells were detectable in the peripheral blood up to 1-7 days after adoptive transfer in most patients with bulk disease but significantly longer (42 days) in a patient with limited disease burden [12]. T cell exhaustion might be a significant cause of shortening persistence. This is confirmed by

 doi:10.20944/preprints202010.0652.v1

discovering the exhausted CAR T cell phenotype in GD2 CAR T cells with low-level tonic signaling [47]. The persistence of infused CAR T cells might be prolonged if the exhaustion was reduced. Thus, several methods have been proposed to increase the persistence of CAR T cells. One such way is the utilization of 2nd and 3rd generation CAR, which improves costimulation after antigen binding (e.g., 4-1BB costimulatory domain) to protect shorten persistence. This development is under investigation for feasibility [13,15,47].

2.2.2. Target selection and on-target, off-tumor effect

Ideally, the target antigen for CAR T cells should have a high expression on cancer cells, a low expression in normal cells, and not associated with oncogenesis [48]. It is known that there are challenges in the way of choosing an optimal CAR T cell target antigen in neuroblastoma is challenging since many target antigens are related to normal peripheral nerves or neural tissue expression. Toxicities caused by particular interactions between the CAR and its target antigen expressed by normal cells termed on-target, off-tumor effects have been reported in previous CAR studies in solid tumors [49-52]. One clinical trial in metastatic colon cancer reported the pulmonary infiltration by CAR T cells that cause a systemic cytokine storm in patients who received HER2-targeted CAR T cell therapy, demonstrating strong evidence of on-target, off-tumor toxicities [51]. On the other hand, there is no such effect in pediatric sarcomas study using anti-HER2 CAR T cells and anti-GD2 CAR T cells in neuroblastoma studying [42,53,54]. This evidence suggested that the variation of antigen density on the different types of cancer is an additional factor to concern during target selection to avoid on-target, off-tumor effects.

2.2.3. Tumor microenvironment

Unlike the remarkable success of CAR T cells in the treatment of hematological malignancies, the efficacy of CAR T cells in neuroblastoma can be obstructed by the immunosuppressive tumor microenvironment (TME), which is a manifest barrier to achieve full effective CAR T cell therapy for solid tumors [55]. Significant factors derived from TME in neuroblastoma that include immunosuppressive cells like tumor-associated macrophages (TAMs), type 2, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) which are attributed to poor result of CAR therapy [16]. Another factor is inhibitory ligand present in the TME, such as PD-L1, the ligand for an inhibitory receptor expressed on activated T cells, named PD-1 [56,57]. Remarkably, this habitual expression of the inhibitory ligand in neuroblastoma can cause the loss of CAR T cells [58]. In addition to inhibitory signals, the availability of soluble factors in TME including galactin 1 and 3, TGF-β, and IL-10 can trigger T cell inhibitory pathways or inhibit T cell function [57,59-62], while secretory HMGB1 may be responsible for Treg differentiation in neuroblastoma TME [63]. Furthermore, there are physical barriers that prevent tumor access of T cells, such as protease fibroblast activation protein (FAP) expressed by tumor-associated stromal fibroblasts, extracellular matrix (ECM), and immunosuppressive tumor vasculature like vascular endothelial growth factor (VEGF) [64,65].

2.3.4. CAR trafficking

Trafficking of CAR cells into solid tumor sites to exert antitumor activity needs to be improved, especially in neuroblastoma. Several chemokines that can mediate immune cell trafficking are generally excreted by tumor or stromal cells like CC- chemokine ligand 17 (CCL17), CCL22, and CCL2 to enhance localization of immune cells [66]. Moreover, suitable trafficking of immune cells, like T cells, can occur when there is an upregulation of a chemokine receptor that is matched to chemokine related trafficking on T cells. However, in a previous study using CAR T cells derived from neuroblastoma patients, low expression of CCR2, a chemokine receptor, was detected [67-69]. Thus, various approaches to generate CAR T cells with an ability to traffic to neuroblastoma sites are underway.

3. Strategies to improve CARs in neuroblastoma

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 October 2020 doi:10.20944/preprints202010.0652.v1

Remarkable the engineering of T lymphocytes to target tumor-associated antigens by forced expression of CARs has been successful against $CD19^{+}$ leukemia [70-72]. Similar results have not yet been achieved against neuroblastoma [73-75]. The key factors that challenge the success of CARs immunotherapies are the immune effector cells, the design of CARs construct, and the intrinsic tumor factors. The proposed strategies for improving CARs efficacy in neuroblastoma therapy are illustrated in **Figure 3**.

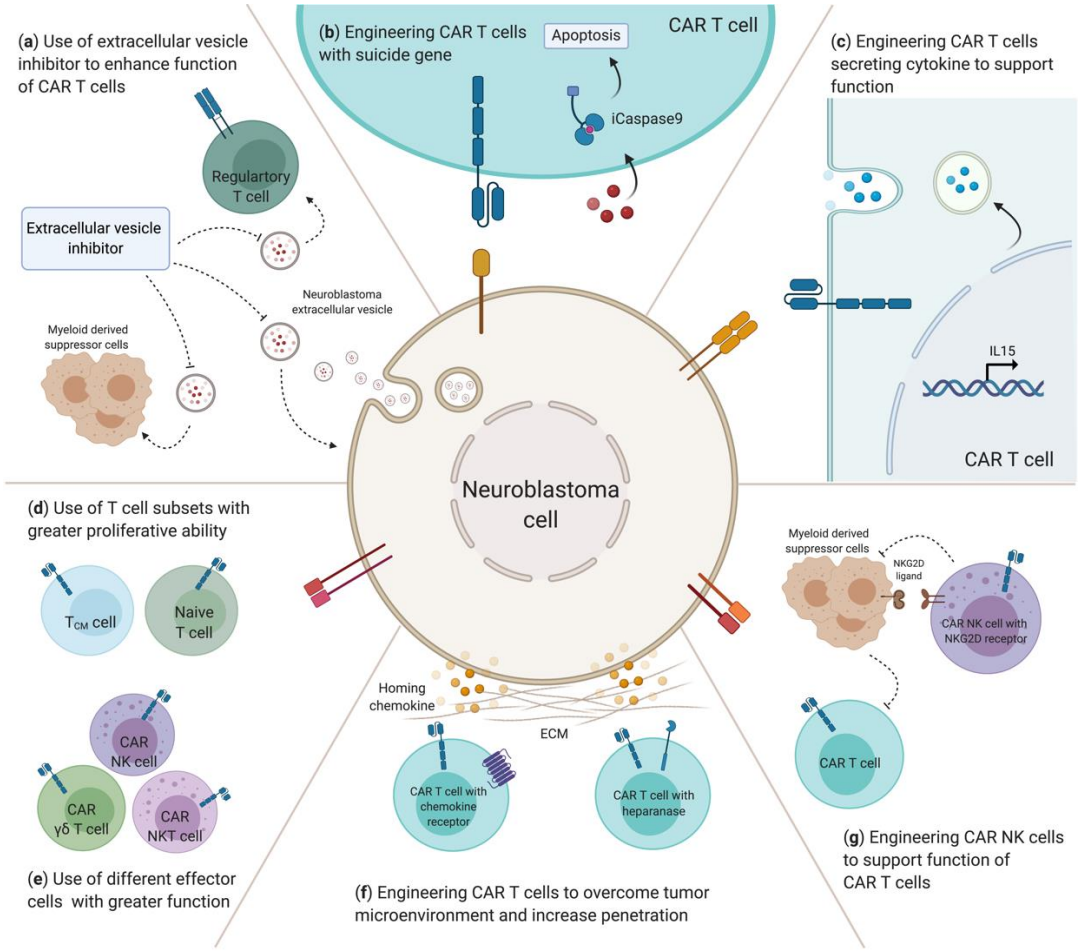

Figure 3. Proposed strategies to improve CARs efficacy against neuroblastoma. To enhance the anti-tumor toxicity of chimeric antigen receptor (CAR) T cells, several strategies can be applied; (**a**) The use of extracellular vesicles (EVs) inhibitor. EVs are cargo ships containing functional molecules that enhance tumor growth and metastasis, and may decoy CAR immunotherapy. Inhibiting secretion of EVs from tumor cells may improve killing efficiency CAR T treatment; (**b**) Engineering CAR T cell with suicide gene. To improve CARS, and any side effects of CARs, fusing iCaspase-9 gene in CARs structure can break T cell activations via induction of apoptosis in CAR T cells; (**c**) Engineering CAR T cells secreting cytokine to support function. IL-15 cytokines showed ability to increase CARs anti-tumor toxicity in both T and NKT cells; (**d**) Use of T cell subsets with greater proliferative ability. Naïve and central memory effector cells have potential to both enhance CARs efficacy and persistency of immune effector cells; (**e**) Use of different effector cells with greater function. For various solid tumors, CARs-derived from NK, NKT or γδT cells showed more penetration into tumor sites and more potent toxicity than CAR T cells; (**f**) Engineering CAR T cells to overcome TME and increase penetration. Supplementing with tumor-enriched chemokine inhibitor or using extracellular matrix remodeling enzyme during treatment might be improve the trafficking and infiltration of CARs T cells; (**g**) Finally, engineering CAR NK cells to support function of CAR T cells.

 doi:10.20944/preprints202010.0652.v1

Inhibition of immunosuppressive cells function in TME via CAR NK cells can enhance homing and efficacy of neuroblastoma-targeted CAR T cell.

3.1 Improving effector immune cells

Solving the CARs T cell obstruction or use of other effector cells might increase CAR's tumor-killing activity on neuroblastoma. Regarding the killing effect against tumor cells, natural killer T (NKT) [76], gamma-delta T (γδT) [77], and natural killer (NK) [78] are the valuable candidates for generating CAR-derived cells. These immune cells have different pros and cons. Thus, choose the proper effector cell for each tumor targets is extremely important. This part will assemble information about these CAR-derived effector cells, some of which are summarized in **Table 2**.

Table 2. Comparison of CAR-derived effector cells for neuroblastoma.

Effector cell	*Ex vivo* expansion	Type of target cells		CAR design		Combination		
		Localized	CTC	Costimulating	Signaling	Cytokine	Effector cell	Signaling inhibitor
T	***	*	***	Dim expression target: CD28 High expression target: 4-1BB	Normally: CD3ζ Decrease CRS: DAP12	IL-2, IL-7, IL-15	NKG2D.CAR NK	anti-PD-1/PD-L1, anti-IL-10, IL-6 inhibitor
NK	**	***	***	CD28, 4-1BB, DNAM1 and 2B4	CD3ζ and DAP12	IL-2, IL-7, IL-21	NB-targeted CAR T	anti-PD-1/PD-L1, anti-TGFβ, anti-IL-10, IL-6 inhibitor
NKT	***	***	***	CD28	CD3ζ and DAP12	IL-2, IL-7, IL-15	N/A	anti-PD-1/PD-L1, anti-IL-10, IL-6 inhibitor
γδT	***	***	***	Both CD28 and 4-1BB	CD3ζ [no data for DAP12]	IL-2, IL-7, IL-15	N/A	anti-PD-1/PD-L1, anti-IL-10, IL-6 inhibitor

Score: *** = high, ** = moderate, * = low; CAR, chirmeric antigen receptor; CTC, circulating tumor cells; DAP12; DNAM1, DNAX accessory Molecule-1; DNAX-activaating protein 12; ECM, extracellular matrix; IL, interleukin; NA, not applicable; NB, neuroblastoma; NK, natural killer cell; NKG2D, natural killer cell receptor D; NKT, natural killer T lymphocyte cell; PD-1, program cell death-1; PD-L1, program cell death-ligand 1; T, T lymphocyte; γδT,gamma delta T lymphocyte.

3.1.1. T lymphocyte

To date, the immunophenotype of T cells is well understood as an essential parameter in safety and efficacy features in CAR T products [74,79]. The ratio of subtype composition, available of naïve and central memory T (T_{CM}) cell population, is essential to increase the therapeutic efficacy of CAR T cells. Shah NN. *et al.*, reported that enriching the $CD4^+$ and $CD8^+$ in starting T cell population before CAR-lentiviral transfection can enhance the efficacy of CAR T cells on neuroblastoma [80]. Also, the 1:1 ratio of subtype composition between $CD4^+$ and $CD8^+$ possibly promotes high efficiency with more safety in patients [75]. Furthermore, another study exhibited an increased percentage of $CD4^+$ T cell and $CD45RO^+CD62L^+$ T_{CM} in CARs treatment of neuroblastoma shown to prolong the persistence of CAR T cells in clinical trials [74]. Interestingly, CAR T cells derived from naïve T cells and T_{CM} cells strongly proliferated ex vivo resulting in 89% of CAR T cell population [81]. Likewise, the procession of CD62L, a standard marker of memory cells, in CARs population is more effective against both hematological malignancy and solid tumors, and promoted longer persistence of peripheral NKT and T cells [82-84]. Because of timing in infiltration into the tumor site, recognizing the tumor antigen, and performing their function, the memory subtype of CAR T cells is exigency [85]. The selection of T cell subtype population during CAR production or before administration to patients is recommended for improving the efficacy of CARs T cell against neuroblastoma. Nonetheless, CAR T cells are mostly detected in peripheral blood but have lower tumor infiltration than other effector cells such as NKT and NK cells. Thus, a combination of other infiltrated-effector cells or ECM remodeling enzymes may enhance tumor infiltration of T cells. In contrast, neuroblastoma-targeted CARs T cells may be more appropriate for eradicating the circulating neuroblastoma cells in patients' blood and lymphatic system.

3.1.2. Natural killer cells

Natural killer (NK) cell, a lymphoid component of the innate immune system, was also applied in CARs immunotherapy against both hematological malignancy and solid tumors [86-88]. NK cells can localize, migrate, and infiltrate into tumor sites to elicit MHC-unrestricted cytotoxicity and proinflammatory cytokines and chemokines against solid tumors [89]. In addition, NK cell showed the antitumor responses via NKG2D cytotoxic adapter molecule DAP10 to eliminate MDSCs, the immunosuppressor cells in neuroblastoma TME. These leading other lymphocytes, such as T cell infiltration into tumor [90], NK cells might requisite proper CARs constructs such as costimulator and/or signaling molecule, which are discussed in the next topic. Several researchers informed that antitumor properties of CARs NK cells were downregulated by suppressive molecules of the TME, such as TGFβ, thereby limiting the tumor-killing capability of NK cells [90,91]. In this direction, a combination with the inhibitor of immunosuppressive molecule (e.g., anti-TGFβ) might increase the antitumor activities of neuroblastoma-targeted CARs NK.

3.1.3. NKT cells

Vα24-invariant natural killer T cells (NKTs) are a subset of innate lymphocytes that share T and NK cells properties [92,93]. The first use of CARs NKT cells against neuroblastoma had been reported since 2014 [76]. Both 2^{nd} and 3^{rd} generation of anti-GD2 CAR NKT cells exhibited potent antitumor activity and significantly presented prolong survival without any graft-versus-host disease in a mouse xenograft model [76]. Unlike T cells, NKT effectively traffic and infiltrate into the tumor site [83] to mediate antitumor activities, inhibit tumor-supportive macrophage, and transactivate the localized NK and $CD8^+$ T cells [94,95]. Additionally, a high percentage of NKT cells in tumor-infiltrating lymphocytes have been detected in neuroblastoma than in patients' peripheral blood [96]. Likewise, Xu X. et al. reported that CARs NKT cells enhanced CARs treatment efficacy through their persistence property and provided the high localization onto the tumor site without significant histopathological toxicity [97]. These suggested that NKT cells are easily infiltrated into neuroblastoma than T lymphocyte. Moreover, the first CAR NKT cell trial is now recruiting

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 October 2020 doi:10.20944/preprints202010.0652.v1

(NCT03294954) [97]. Neuroblastoma-targeted CARs NKT cells are noticeable as the potential effector cells against neuroblastoma and other solid tumors. Of note, an exhausting phenotype of NKTs upon in vitro stimulation is associated with the upregulation expression of PD-1 and TIM-3 [83]. Thus, CAR NKT cells should be used in combination with anti-PD-1 or anti-TIM-3 to generate an intense tumor-killing effect.

3.1.4. γδT cells

γδT cells are innate T lymphocytes that independently recognize MHC targets, have a natural killing activity against pathogens, and respond to the cells [98-100]. Normally, neuroblastoma mostly escapes from the immune system via the downregulation of MHC class I molecule [96]. Thus γδT cells have the potential to solve this problem. Additionally, γδT cells have been found in neuroblastoma tumors [96] and a wide variety of tissues [101]. These cells can differentiate into professional antigen-presenting cells (pAPCs), which present the antigenic fragments for $CD4^+$ and $CD8^+$ αβT cells [102]. γδT cells expressing the Vγ9Vδ2 TCR is a common subset that can be activated and proliferated ex vivo for more than 1000 fold expansion [103]. Although γδT cells exhaustion is associated with PD-1, an immune checkpoints molecule [104], γδT cells have lower expression of PD-1 compared to αβT cells, thereby recognized as a promising effector cell for CAR immunotherapy. Like T cells, the naïve and memory phenotype of γδT cells sufficiently survive to promote persistence of CAR γδT cells in the recipients without decreasing the killing activity against tumor cells [98]. From these properties, γδT cell is a potential candidate for using as effector cells in adoptive CAR immunotherapy against neuroblastoma.

3.2 Modification of CARs constructs

The CAR construct design is one of the key features that affect the kinetics of expansion and the duration of persistence. CARs are recombinant receptors responsible for both antigen-binding and activation functions for immune cells [105]. The 2^{nd} generation CAR offers a clinical benefit via both TCR stimulatory domain (CD3z) and a single-co-stimulatory domain [80]. Recently, FDA-approved products contain either CD28 or 4-1BB (CD137) costimulatory domain [70]. Choosing the appropriate combination of costimulatory ligands, chimeric costimulatory receptors, or cytokines in CARs structure to each objective immune cell may differently enhance the effector cell function, proliferation, and survival [105]. The well-design of CAR structures can overcome the significant barrier of CARs in cancer immunotherapy.

3.2.1. Hinge and transmembrane domain

The access of CAR to the target antigen is depended on their flexibility and length of hinge and transmembrane domain [106]. In the case of anti-CD19 CAR T cells, human CD8α hinge and transmembrane molecule promoted efficacies indifferently against tumor xenograft but induce lower cytokine levels compared to CD28 hinge and transmembrane [107,108]. These confirmed that the length and composition of hinge provided antigen binding and signaling cascade through the CAR T cells. Accordingly, the CD8α hinge and transmembrane domain may be an excellent choice to generate neuroblastoma-targeted CARs. However, further studies on this topic are required to pursue a suitable composition that offers the highest tumor-killing efficacy but bestows the patients' low or no side effect.

3.2.2. Costimulatory domains

Costimulator molecules play a critical role in developing, activation, and functional response of effector immune cells [83]. A set of costimulatory receptors that noticeable in human T cells are including; CD28, 4-1BB, and OX40 [83], while the costimulators for activating NK cells are composed of CD28, 4-1BB, 2B4, and NKG2D [109,110]. For creating CAR T cells, CD28 has more potent signaling through T cell activation with a short persistence, while 4-1BB provides a less potent signal but longer persisted [111]. To address cytokine releasing syndrome [CRS], CD28 co-stimulator is worthy of the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 October 2020 doi:10.20944/preprints202010.0652.v1

dim target antigen, whereas 4-1BB is suitable for the high density of target [108]. Unlike T cells, CD28 costimulator is suitable for enhancing proliferation, cancer-killing function, and persistence of NKT cells. Even though CD28 and 4-1BB prevent the loss of CD62L expression that is important to prolong the survival of NKT cells [83], the CD28 costimulator also forceful the CAR NKT cell to massive expansion while 4-1BB induce excessive activation leading to cell death [97]. For CAR γδT cell, both CD28 and 4-1BB [112] were applied as the costimulator [113,114]. However, data on the comparison and optimization of costimulators for CAR γδT cells are scant. For NK cells, CD28 is mostly used as a costimulator. Oelsner S, et al. suggested that CD19-CAR NK with CD28 costimulator showed better performance against B-cell malignancy than 4-1BB [115]. Also, fusing DNAM1 and 2B4 costimulatory domains in CAR NK design can enhance cytotoxicity against hepatocellular cancer cells in vitro [116].

3.2.3. Signaling-transducing domain

The CD3ζ is commonly used as the signaling molecule in CAR constructs to activate T and NK cells functions for both hematologic malignancy and solid tumors [71,80,107,117]. Immunotherapy resistance and CRS are associated with highly active CD3ζ-activated CAR T cells [118,119]. Implementing other activation domains in CAR design should be considered an alternative to provide a robust clinical advantage in relieving the risk of CRS and overcoming these barriers.

DNAX activation protein of 12 kDa (DAP12), an immunotyrosine-based activation motifs-containing adaptor, is constitutively expressed in NK cells while expressed in a subset of human T cells [120]. The first use of DAP12 as the alternative cytoplasmic domain in CAR design was emerged in 2015 [121]. The chimeric target receptor fusing with the transmembrane and cytoplasmic domains of KIR2DS2, a stimulatory killer immunoglobulin-like receptor, and DAP12 (KIR-CAR DAP12) triggered robust antigen-specific proliferation, effector function, and enhanced antitumor activity in leukemia cell lines [121]. Although only a few studies used the DAP12 as the newly activation domain in CAR-modified T cells, the low cytokine markers of CRS could be observed in DAP12-based CARs preclinical studies [118]. Comparing to CD3ζ-based 2nd generation CARs, T cells modified with the natural killer group 2D (NKG2D) ectodomain combining with 4-1BB and the DAP12 signaling domain release lower levels of interferon-gamma (IFN-γ), Tumor necrosis factor-alpha (TNF-α), and interleukin-2 (IL-2) during tumor cell lysis without significant difference of tumor-killing effect both in vitro and in vivo [118]. DAP12 is also involved in signal transduction of activating NK cells [122]. DAP12-based CARs NK cells could eradicate neuroblastoma in the mouse xenograft model [122]. This DAP12 might be the new brilliant signaling molecule for high efficacy with more safety in CARs development

3.2.4. Others generation of CARs structures

Although the 2nd generation of CARs is mostly used in neuroblastoma clinical trials, the 4th generation CAR has been developed. With neuroblastoma-targeted antigen incorporated multiple costimulatory molecules and a suicide gene inducible caspase9 (iCasp9), the success of 4th CAR T cells against MYCN amplification neuroblastoma has been reported [123]. This 4th generation CAR exhibited higher tumor-killing activity than the 3rd generation CAR with a safety profile, leading to 4 years survival of a neuroblastoma patient after 4th generation CAR treatment [123]. Successful of this 4th generation CAR T cells is explored to the new era treatment for neuroblastoma and other solid tumors.

3.3 Overcoming TME

The intrinsic tumor factors, including extracellular matrix (ECM) and the TME, are the critical challenges for CAR immunotherapy against neuroblastoma [124]. The counteracts for these tumor escape mechanisms are showed in this topic.

3.3.1. ECM: trafficking and infiltration of effector cells

 doi:10.20944/preprints202010.0652.v1

The low T cell infiltration has been observed in neuroblastoma so-called immunologically "cold" tumors [125]. Various studies demonstrated that poor trafficking and limited CAR T cell persistence to solid tumors are significant burdens in CAR immunotherapy [126]. Tumor chemokines and their receptor play an essential role in TME [127]. Chemokines also mediate proliferation, infiltration, and persistence of leukocytes [128], which affected tumor control [129]. Aberrant expression of tumor chemokine-chemokine receptor network affected several tumor biological functions, i.e., stimulating tumor cell proliferation, inducing tumor angiogenesis [130], enrolling inhibitory immune cells [131], preventing recruitment of effector lymphocytes [132], and resisting their antitumor response [133], leading to cancer immune escape [134]. Increased trafficking ability of CAR T cells via presenting the matched chemokine receptors or adding therapeutic agents against tumor-enriched chemokines may be a practical strategy for neuroblastoma and other solid tumor treatments [135]. For example, in hepatocellular carcinoma in which the high expression level of CXCR2 ligand was observed, Liu G, et al., confirmed that using CXCR2 CAR T cells could enhance in vivo trafficking and tumor cytotoxicity [127]. Additionally, the fusion of IL-7 and CCL19 on CAR structure showed increased infiltration and exhibited high antitumor activity of CAR T cells [136]. Moreover, given the prevalence of IL-8 production in human cancer [137], this approach may find broad applicability in the potentiation of CAR T cell immunotherapy for solid tumors. Accordingly, the tumor chemokine-chemokine receptor network in neuroblastoma should be identified. Studies showed that VEGF, IL-6, and IL-10 receptor (IL-10R) in neuroblastoma were associated with poor outcomes [138-140]. Thus, the fusion of anti-VEGF, anti-IL-6, or anti-IL-10R into CAR structure may also be effective against neuroblastoma.

3.3.2. Myeloid-derived suppressor cells (MDSCs)

MDSCs are the immunosuppressive immune cells found in TME [141,142]. These MDSCs enhance tumor growth and suppress the infiltration, proliferation, and tumor-killing activity of CAR T cells [143]. These cells also express PD-L1 themselves, which contribute to immune escape in various solid tumors [144]. Researchers found that NKG2D ligand, a cytotoxicity receptor activated by non-classical MHC molecules, is overexpressed on these tumor-infiltrating MDSCs [145,146]. Combination with NKG2D-CAR NK cells enhanced tumor infiltration and expansion of CAR T cells at tumor sites and prolonged CAR T cell survival compared to single treatment [90,147]. The mixed NKG2D-CAR NK and neuroblastoma-targeted CAR T cell treatment may safely enhance antitumor activity against neuroblastoma that are supported and protected by MDSCs.

3.3.3. Tumor extracellular vesicles

Extracellular vesicles (EVs) are the lipid bilayer vesicle released from the cells [148], which can be classified into three major groups; exosome, microvesicles, and apoptotic bodies, based on their size [149]. Like cargo, EVs containing soluble proteins such as growth factors, cytokines, and chemokines [150], lipids, metabolites, nucleic acids, including regulatory microRNAs (miRs) [149,151]. Several studies reported that EVs play a crucial role in tumor-tumor [152,153] and tumor-immune cell communications [154] and affect tumor cell phenotype and metastatic potential [155-157]. Neuroblastoma cells release EVs in their extracellular space [150]. Previous studies showed that neuroblastoma-EVs induced the production of pro-tumorigenic cytokines and chemokines such as IL-6, IL-8, VEGF, and CCL2 by MSCs [158]. Additionally, neuroblastoma-EVs also trigger a proinflammatory response in monocytes and promote neuroblastoma chemoresistance [159]. Future studies aim to reveal the exact roles of EVs in the communication between neuroblastoma and TME is required to decipher a novel treatment strategy against neuroblastoma TME.

3.4 Combination of CARs

3.4.1. PD-1/PDL-1

Program cell death 1 (PD-1 or PDCD1) is a coinhibitory receptor expressed by all CAR effector cells, including $\alpha\beta$T [160], $\gamma\delta$T [113], NKT [97], and NK cells [104]. PD-1 binding to its ligand, PD-L1

 doi:10.20944/preprints202010.0652.v1

[or CD274], leading to a decrease in effector cell receptor downstream signaling and diminishing T cell activation [161]. Combining with PD-1/PD-L1 inhibitor is recommended for turning neuroblastoma microenvironment to response to immunological treatment. These might enhance the efficacy of CAR therapy against neuroblastoma as well [162,163].

3.4.2. Cytokines

Another approach to improve the potency of CARs is to genetically modify the effector cells secreting pro-inflammatory or pro-proliferative cytokines, aiming to strengthen effector function, proliferation, persistence, and to alter the TME [105,164]. The cytokines IFN-γ, IL-2, IL-7, IL-12, IL-15, IL-18, IL-21, and IL-27, promoted neuroblastoma regression via enhancing the efficacy of effector cells, whereas VEGF, IL-6, IL-10 induced neuroblastoma progression through their immunosuppressive activities. Several studies reported that increasing the proinflammatory chemokine IL-8 levels in patients was related to poor prognosis in neuroblastoma and other solid tumors [165,166]. This information is useful for the new design of CAR construct or combination therapy to enhance the efficacy of CARs against neuroblastoma.

To date, IL-15 cytokine has been studied for its ability to enhance CAR antitumor. IL-15 plays a crucial role in T and NKT cells [97,167] via enhanced CAR antitumor activity and survival in peripheral blood and tumor tissue [167]. This study suggested that IL-15 conjugated CD28-CAR structure reduced the exhausting marker, improved the persistence and the tumor-killing activity of NKT-established CARs. Recently, Xin Xu, et al., utilized mice bearing neuroblastoma xenografts to demonstrate that GD2-CAR IL-15 NKT cells enhanced in vivo persistence, increased localization to tumor sites, and improved tumor control as compared with GD2-CAR NKT cells [97]. Interestingly, IL-15 conjugated GD2 CAR T cells reduced the expression of the PD-1 receptor [167]. These characteristics have made IL-15 a promising candidate for CAR T (or NKT) design for neuroblastoma immunotherapy.

4. Future perspective

The strategies to improve CAR T cells using in neuroblastoma are mostly concerned with increasing the antitumor activity and persistence of the infused CAR T cells. Therefore, discovering new tumor antigens that are more restricted to neuroblastoma with less neuronal toxicity is very necessary. The evaluation of new target antigens such as B7H3 or o-acetyl-GD2 (oaGD2) is on-going [168,169]. Researchers can also modify the construct of CAR T cells to be more effective by adding new costimulatory endodomains such as 4-1BB or ICOS, which can be seen in the 4th generation of CAR therapy [170,171]. Seeking a way to overcome the immunosuppressive TME in neuroblastoma is a critical challenge in CAR studies. Various trials of CAR T cell therapy in neuroblastoma, which were relied on the strategies mentioned above, are underway validating to improve outcomes. Alternatively, administration of antitumor related cytokine might enhance immune response and amplify the direct neuroblastoma cell killing of CAR cells in complex TME. For example, IL-15 has been used to improve *in vivo* persistence and antitumor activity against neuroblastoma. Hence, another area of interest in CAR structure design is the induction of cytokine expression.

Given all these findings, understanding the mechanism of TME affects the resistance of CAR cells and inhibits the *in vivo* antitumor function is vastly relevant. The exploration of exosomal miRs or exosome decoy released within neuroblastoma TME may be essential alternatives to embrace the barriers posed by the TME. Furthermore, by understanding molecular mechanisms within the TME, researchers can efficiently engineer molecular targeting CAR T cells to restore TME resistance sensitivity.

Lastly, a more practical approach for CAR T cell manufacturing is still in need. The ability to manufacture prompt quantities of an efficacious cell product is required for neuroblastoma patients with varied conditions. In the end, good manufacturing might help to create highly active CAR T cells for patients with relapsed disease in time.

5. Conclusions

In summary, immunotherapy using CARs in neuroblastoma treatment has shown promising efficacy and safety. To utilize the therapeutic benefits of CARs as the first line of immunotherapeutic approach in patients with high risk or relapsed neuroblastoma, several approaches have been raised further to augment the antitumor function of CAR-modified cells *in vivo*. However, unlike the remarkable success in hematological malignancies, various barriers restrict the effective use of this CAR treatment in clinical trials, as indicated earlier. The strategies provided in this review may offer suitable approaches to address the benefits of CAR therapy in neuroblastoma.

Author Contributions: Conceptualization, S.C.; writing—original draft preparation, P.S., K.A.; writing—review and editing, U.A., S.C., S.H.; visualization, P.S., K.A., S.C.; supervision, U.A., S.H.; funding acquisition, S.H.

Funding: This study was funded by the Research Chair Grant from the National Science and Technology Development Agency of Thailand, grant number FDA-CO-2559-3325-TH.

Acknowledgments: This study was supported by the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand. S.C. was financially supported by the Faculty Staff Development Program of Faculty of Medicine Ramathibodi Hospital, Mahidol University, for his research activities. All figures were created with BioRender.com.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Ahmed, A.A.; Zhang, L.; Reddivalla, N.; Hetherington, M. Neuroblastoma in children: Update on clinicopathologic and genetic prognostic factors. *Pediatr Hematol Oncol* **2017**, *34*, 165-185.
2. Moreno, L.; Rubie, H.; Varo, A.; Le Deley, M.C.; Amoroso, L.; Chevance, A.; Garaventa, A.; Gambart, M.; Bautista, F.; Valteau-Couanet, D., et al. Outcome of children with relapsed or refractory neuroblastoma: A meta-analysis of ITCC/SIOPEN European phase II clinical trials. *Pediatr Blood Cancer* **2017**, *64*, 25-31.
3. Smith, M.A.; Altekruse, S.F.; Adamson, P.C.; Reaman, G.H.; Seibel, N.L. Declining childhood and adolescent cancer mortality. *Cancer* **2014**, *120*, 2497-2506.
4. Ries, L.A.G.; SEER Program (National Cancer Institute (U.S.)). *Cancer incidence and survival among children and adolescents : United States SEER program 1975-1995 /[edited by Lynn A. Gloecker Ries ... et al.]*; National Cancer Institute, SEER Program: Bethesda, MD, 1999; pp. vi, 182 p.
5. Matthay, K.K.; Maris, J.M.; Schleiermacher, G.; Nakagawara, A.; Mackall, C.L.; Diller, L.; Weiss, W.A. Neuroblastoma. *Nat Rev Dis Primers* **2016**, *2*, 16078.
6. Brodeur, G.M. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer* **2003**, *3*, 203-216.
7. Huibregtse, K.E.; Vo, K.T.; DuBois, S.G.; Fetzko, S.; Neuhaus, J.; Batra, V.; Maris, J.M.; Weiss, B.; Marachelian, A.; Yanik, G.A., et al. Incidence and risk factors for secondary malignancy in patients with neuroblastoma after treatment with (131)I-metaiodobenzylguanidine. *Eur J Cancer* **2016**, *66*, 144-152.
8. Li, R.; Polishchuk, A.; DuBois, S.; Hawkins, R.; Lee, S.W.; Bagatell, R.; Shusterman, S.; Hill-Kayser, C.; Al-Sayegh, H.; Diller, L., et al. Patterns of Relapse in High-Risk Neuroblastoma Patients Treated With and Without Total Body Irradiation. *Int J Radiat Oncol Biol Phys* **2017**, *97*, 270-277.
9. Cheung, I.Y.; Kushner, B.H.; Modak, S.; Basu, E.M.; Roberts, S.S.; Cheung, N.V. Phase I trial of anti-GD2 monoclonal antibody hu3F8 plus GM-CSF: Impact of body weight, immunogenicity and anti-GD2 response on pharmacokinetics and survival. *Oncoimmunology* **2017**, *6*, e1358331.
10. Yu, A.L.; Gilman, A.L.; Ozkaynak, M.F.; London, W.B.; Kreissman, S.G.; Chen, H.X.; Smith, M.; Anderson, B.; Villablanca, J.G.; Matthay, K.K., et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med* **2010**, *363*, 1324-1334.
11. Kushner, B.H.; Ostrovnaya, I.; Cheung, I.Y.; Kuk, D.; Kramer, K.; Modak, S.; Yataghene, K.; Cheung, N.K. Prolonged progression-free survival after consolidating second or later remissions of neuroblastoma with Anti-GD2 immunotherapy and isotretinoin: a prospective Phase II study. *Oncoimmunology* **2015**, *4*, e1016704.
12. Park, J.R.; Digiusto, D.L.; Slovak, M.; Wright, C.; Naranjo, A.; Wagner, J.; Meechoovet, H.B.; Bautista, C.; Chang, W.C.; Ostberg, J.R., et al. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. *Mol Ther* **2007**, *15*, 825-833.
13. Gardner, R.A.; Finney, O.; Annesley, C.; Brakke, H.; Summers, C.; Leger, K.; Bleakley, M.; Brown, C.; Mgebroff, S.; Kelly-Spratt, K.S., et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. *Blood* **2017**, *129*, 3322-3331.

14. Lee, D.W.; Kochenderfer, J.N.; Stetler-Stevenson, M.; Cui, Y.K.; Delbrook, C.; Feldman, S.A.; Fry, T.J.; Orentas, R.; Sabatino, M.; Shah, N.N., et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* **2015**, *385*, 517-528.
15. Maude, S.L.; Frey, N.; Shaw, P.A.; Aplenc, R.; Barrett, D.M.; Bunin, N.J.; Chew, A.; Gonzalez, V.E.; Zheng, Z.; Lacey, S.F., et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* **2014**, *371*, 1507-1517.
16. Asgharzadeh, S.; Salo, J.A.; Ji, L.; Oberthuer, A.; Fischer, M.; Berthold, F.; Hadjidaniel, M.; Liu, C.W.; Metelitsa, L.S.; Pique-Regi, R., et al. Clinical significance of tumor-associated inflammatory cells in metastatic neuroblastoma. *J Clin Oncol* **2012**, *30*, 3525-3532.
17. Heczey, A.; Louis, C.U. Advances in chimeric antigen receptor immunotherapy for neuroblastoma. *Discov Med* **2013**, *16*, 287-294.
18. Pule, M.A.; Savoldo, B.; Myers, G.D.; Rossig, C.; Russell, H.V.; Dotti, G.; Huls, M.H.; Liu, E.; Gee, A.P.; Mei, Z., et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat Med* **2008**, *14*, 1264-1270.
19. Savoldo, B.; Dotti, G. Chimeric antigen receptors (CARs) from bench-to-bedside. *Immunol Lett* **2013**, *155*, 40-42.
20. Mosse, Y.P.; Laudenslager, M.; Longo, L.; Cole, K.A.; Wood, A.; Attiyeh, E.F.; Laquaglia, M.J.; Sennett, R.; Lynch, J.E.; Perri, P., et al. Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* **2008**, *455*, 930-935.
21. De Brouwer, S.; De Preter, K.; Kumps, C.; Zabrocki, P.; Porcu, M.; Westerhout, E.M.; Lakeman, A.; Vandesompele, J.; Hoebeeck, J.; Van Maerken, T., et al. Meta-analysis of neuroblastomas reveals a skewed ALK mutation spectrum in tumors with MYCN amplification. *Clin Cancer Res* **2010**, *16*, 4353-4362.
22. Moog-Lutz, C.; Degoutin, J.; Gouzi, J.Y.; Frobert, Y.; Brunet-de Carvalho, N.; Bureau, J.; Creminon, C.; Vigny, M. Activation and inhibition of anaplastic lymphoma kinase receptor tyrosine kinase by monoclonal antibodies and absence of agonist activity of pleiotrophin. *J Biol Chem* **2005**, *280*, 26039-26048.
23. Walker, A.J.; Majzner, R.G.; Zhang, L.; Wanhainen, K.; Long, A.H.; Nguyen, S.M.; Lopomo, P.; Vigny, M.; Fry, T.J.; Orentas, R.J., et al. Tumor Antigen and Receptor Densities Regulate Efficacy of a Chimeric Antigen Receptor Targeting Anaplastic Lymphoma Kinase. *Mol Ther* **2017**, *25*, 2189-2201.
24. Li, N.; Fu, H.; Hewitt, S.M.; Dimitrov, D.S.; Ho, M. Therapeutically targeting glypican-2 via single-domain antibody-based chimeric antigen receptors and immunotoxins in neuroblastoma. *Proc Natl Acad Sci U S A* **2017**, *114*, E6623-E6631.
25. Baral, A.; Ye, H.X.; Jiang, P.C.; Yao, Y.; Mao, Y. B7-H3 and B7-H1 expression in cerebral spinal fluid and tumor tissue correlates with the malignancy grade of glioma patients. *Oncol Lett* **2014**, *8*, 1195-1201.
26. Zhou, Z.; Luther, N.; Ibrahim, G.M.; Hawkins, C.; Vibhakar, R.; Handler, M.H.; Souweidane, M.M. B7-H3, a potential therapeutic target, is expressed in diffuse intrinsic pontine glioma. *J Neurooncol* **2013**, *111*, 257-264.
27. Du, H.; Hirabayashi, K.; Ahn, S.; Kren, N.P.; Montgomery, S.A.; Wang, X.; Tiruthani, K.; Mirlekar, B.; Michaud, D.; Greene, K., et al. Antitumor Responses in the Absence of Toxicity in Solid Tumors by Targeting B7-H3 via Chimeric Antigen Receptor T Cells. *Cancer Cell* **2019**, *35*, 221-237 e228.
28. Majzner, R.G.; Theruvath, J.L.; Nellan, A.; Heitzeneder, S.; Cui, Y.; Mount, C.W.; Rietberg, S.P.; Linde, M.H.; Xu, P.; Rota, C., et al. CAR T Cells Targeting B7-H3, a Pan-Cancer Antigen, Demonstrate Potent Preclinical Activity Against Pediatric Solid Tumors and Brain Tumors. *Clin Cancer Res* **2019**, *25*, 2560-2574.
29. Crossland, D.L.; Denning, W.L.; Ang, S.; Olivares, S.; Mi, T.; Switzer, K.; Singh, H.; Huls, H.; Gold, K.S.; Glisson, B.S., et al. Antitumor activity of CD56-chimeric antigen receptor T cells in neuroblastoma and SCLC models. *Oncogene* **2018**, *37*, 3686-3697.
30. Zeromski, J.; Nyczak, E.; Dyszkiewicz, W. Significance of cell adhesion molecules, CD56/NCAM in particular, in human tumor growth and spreading. *Folia Histochem Cytobiol* **2001**, *39 Suppl 2*, 36-37.
31. Singh, N.; Kulikovskaya, I.; Barrett, D.M.; Binder-Scholl, G.; Jakobsen, B.; Martinez, D.; Pawel, B.; June, C.H.; Kalos, M.D.; Grupp, S.A. T cells targeting NY-ESO-1 demonstrate efficacy against disseminated neuroblastoma. *Oncoimmunology* **2016**, *5*, e1040216.
32. Spel, L.; Boelens, J.J.; van der Steen, D.M.; Blokland, N.J.; van Noesel, M.M.; Molenaar, J.J.; Heemskerk, M.H.; Boes, M.; Nierkens, S. Natural killer cells facilitate PRAME-specific T-cell reactivity against neuroblastoma. *Oncotarget* **2015**, *6*, 35770-35781.
33. Richards, R.M.; Sotillo, E.; Majzner, R.G. CAR T Cell Therapy for Neuroblastoma. *Front Immunol* **2018**, *9*, 2380.
34. Arlt, M.J.; Novak-Hofer, I.; Gast, D.; Gschwend, V.; Moldenhauer, G.; Grunberg, J.; Honer, M.; Schubiger, P.A.; Altevogt, P.; Kruger, A. Efficient inhibition of intra-peritoneal tumor growth and dissemination of

human ovarian carcinoma cells in nude mice by anti-L1-cell adhesion molecule monoclonal antibody treatment. *Cancer Res* **2006**, *66*, 936-943.

35. Wang, X.; Chang, W.C.; Wong, C.W.; Colcher, D.; Sherman, M.; Ostberg, J.R.; Forman, S.J.; Riddell, S.R.; Jensen, M.C. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. *Blood* **2011**, *118*, 1255-1263.
36. Kunkele, A.; Taraseviciute, A.; Finn, L.S.; Johnson, A.J.; Berger, C.; Finney, O.; Chang, C.A.; Rolczynski, L.S.; Brown, C.; Mgebroff, S., et al. Preclinical Assessment of CD171-Directed CAR T-cell Adoptive Therapy for Childhood Neuroblastoma: CE7 Epitope Target Safety and Product Manufacturing Feasibility. *Clin Cancer Res* **2017**, *23*, 466-477.
37. Rossig, C.; Bollard, C.M.; Nuchtern, J.G.; Merchant, D.A.; Brenner, M.K. Targeting of G(D2)-positive tumor cells by human T lymphocytes engineered to express chimeric T-cell receptor genes. *Int J Cancer* **2001**, *94*, 228-236.
38. Suzuki, M.; Cheung, N.K.V. Disialoganglioside GD2 as a therapeutic target for human diseases. *Expert Opin Ther Tar* **2015**, *19*, 349-362.
39. Cheung, N.K.; Cheung, I.Y.; Kramer, K.; Modak, S.; Kuk, D.; Pandit-Taskar, N.; Chamberlain, E.; Ostrovnaya, I.; Kushner, B.H. Key role for myeloid cells: phase II results of anti-G(D2) antibody 3F8 plus granulocyte-macrophage colony-stimulating factor for chemoresistant osteomedullary neuroblastoma. *Int J Cancer* **2014**, *135*, 2199-2205.
40. Gilman, A.L.; Ozkaynak, M.F.; Matthay, K.K.; Krailo, M.; Yu, A.L.; Gan, J.; Sternberg, A.; Hank, J.A.; Seeger, R.; Reaman, G.H., et al. Phase I study of ch14.18 with granulocyte-macrophage colony-stimulating factor and interleukin-2 in children with neuroblastoma after autologous bone marrow transplantation or stem-cell rescue: a report from the Children's Oncology Group. *J Clin Oncol* **2009**, *27*, 85-91.
41. Ozkaynak, M.F.; Sondel, P.M.; Krailo, M.D.; Gan, J.; Javorsky, B.; Reisfeld, R.A.; Matthay, K.K.; Reaman, G.H.; Seeger, R.C. Phase I study of chimeric human/murine anti-ganglioside G(D2) monoclonal antibody (ch14.18) with granulocyte-macrophage colony-stimulating factor in children with neuroblastoma immediately after hematopoietic stem-cell transplantation: a Children's Cancer Group Study. *J Clin Oncol* **2000**, *18*, 4077-4085.
42. Straathof, K.; Flutter, B.; Wallace, R.; Thomas, S.; Cheung, G.; Collura, A.; Gileadi, T.; Barton, J.; Wright, G.; Inglott, S., et al. A Cancer Research UK phase I trial of anti-GD2 chimeric antigen receptor (CAR) transduced T-cells (1RG-CART) in patients with relapsed or refractory neuroblastoma. *Cancer Research* **2018**, *78*.
43. Pule, M.A.; Straathof, K.C.; Dotti, G.; Heslop, H.E.; Rooney, C.M.; Brenner, M.K. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. *Molecular Therapy* **2005**, *12*, 933-941.
44. Heczey, A.; Louis, C.U.; Savoldo, B.; Dakhova, O.; Durett, A.; Grilley, B.; Liu, H.; Wu, M.F.F.; Mei, Z.Y.; Gee, A., et al. CAR T Cells Administered in Combination with Lymphodepletion and PD-1 Inhibition to Patients with Neuroblastoma. *Molecular Therapy* **2017**, *25*, 2214-2224.
45. Louis, C.U.; Savoldo, B.; Dotti, G.; Pule, M.; Yvon, E.; Myers, G.D.; Rossig, C.; Russell, H.V.; Diouf, O.; Liu, E.L., et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood* **2011**, *118*, 6050-6056.
46. Robbins, P.F.; Dudley, M.E.; Wunderlich, J.; El-Gamil, M.; Li, Y.F.; Zhou, J.; Huang, J.; Powell, D.J., Jr.; Rosenberg, S.A. Cutting edge: persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. *J Immunol* **2004**, *173*, 7125-7130.
47. Long, A.H.; Haso, W.M.; Shern, J.F.; Wanhainen, K.M.; Murgai, M.; Ingaramo, M.; Smith, J.P.; Walker, A.J.; Kohler, M.E.; Venkateshwara, V.R., et al. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. *Nat Med* **2015**, *21*, 581-590.
48. Himoudi, N.; Yan, M.Y.; Papanastasiou, A.; Anderson, J. MYCN as a target for cancer immunotherapy. *Cancer Immunol Immun* **2008**, *57*, 693-700.
49. Ahmed, N.; Brawley, V.S.; Hegde, M.; Robertson, C.; Ghazi, A.; Gerken, C.; Liu, E.; Dakhova, O.; Ashoori, A.; Corder, A., et al. Human Epidermal Growth Factor Receptor 2 (HER2) -Specific Chimeric Antigen Receptor-Modified T Cells for the Immunotherapy of HER2-Positive Sarcoma. *J Clin Oncol* **2015**, *33*, 1688-1696.
50. Goff, S.L.; Morgan, R.A.; Yang, J.C.; Sherry, R.M.; Robbins, P.F.; Restifo, N.P.; Feldman, S.A.; Lu, Y.C.; Lu, L.; Zheng, Z., et al. Pilot Trial of Adoptive Transfer of Chimeric Antigen Receptor-transduced T Cells Targeting EGFRvIII in Patients With Glioblastoma. *J Immunother* **2019**, *42*, 126-135.
51. Morgan, R.A.; Yang, J.C.; Kitano, M.; Dudley, M.E.; Laurencot, C.M.; Rosenberg, S.A. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* **2010**, *18*, 843-851.

52. Thistlethwaite, F.C.; Gilham, D.E.; Guest, R.D.; Rothwell, D.G.; Pillai, M.; Burt, D.J.; Byatte, A.J.; Kirillova, N.; Valle, J.W.; Sharma, S.K., et al. The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. *Cancer Immunol Immunother* **2017**, *66*, 1425-1436.
53. Hegde, M.; DeRenzo, C.C.; Zhang, H.M.; Mata, M.; Gerken, C.; Shree, A.; Yi, Z.Z.; Brawley, V.; Dakhova, O.; Wu, M.F., et al. Expansion of HER2-CAR T cells after lymphodepletion and clinical responses in patients with advanced sarcoma. *Journal of Clinical Oncology* **2017**, *35*.
54. Navai, S.A.; Derenzo, C.; Joseph, S.; Sanber, K.; Byrd, T.; Zhang, H.M.; Mata, M.; Gerken, C.; Shree, A.; Mathew, P.R., et al. Administration of HER2-CAR T cells after lymphodepletion safely improves T cell expansion and induces clinical responses in patients with advanced sarcomas. *Cancer Research* **2019**, *79*.
55. Mina, M.; Boldrini, R.; Citti, A.; Romania, P.; D'Alicandro, V.; De Ioris, M.; Castellano, A.; Furlanello, C.; Locatelli, F.; Fruci, D. Tumor-infiltrating T lymphocytes improve clinical outcome of therapy-resistant neuroblastoma. *Oncoimmunology* **2015**, *4*, e1019981.
56. Majzner, R.G.; Simon, J.S.; Grosso, J.F.; Martinez, D.; Pawel, B.R.; Santi, M.; Merchant, M.S.; Geoerger, B.; Hezam, I.; Marty, V., et al. Assessment of programmed death-ligand 1 expression and tumor-associated immune cells in pediatric cancer tissues. *Cancer* **2017**, *123*, 3807-3815.
57. Pardoll, D.M. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* **2012**, *12*, 252-264.
58. Moon, E.K.; Wang, L.C.; Dolfi, D.V.; Wilson, C.B.; Ranganathan, R.; Sun, J.; Kapoor, V.; Scholler, J.; Pure, E.; Milone, M.C., et al. Multifactorial T-cell hypofunction that is reversible can limit the efficacy of chimeric antigen receptor-transduced human T cells in solid tumors. *Clin Cancer Res* **2014**, *20*, 4262-4273.
59. Dwivedi, V.P.; Tousif, S.; Bhattacharya, D.; Prasad, D.V.; Van Kaer, L.; Das, J.; Das, G. Transforming growth factor-beta protein inversely regulates in vivo differentiation of interleukin-17 (IL-17)-producing CD4+ and CD8+ T cells. *J Biol Chem* **2012**, *287*, 2943-2947.
60. Seo, N.; Hayakawa, S.; Takigawa, M.; Tokura, Y. Interleukin-10 expressed at early tumour sites induces subsequent generation of CD4(+) T-regulatory cells and systemic collapse of antitumour immunity. *Immunology* **2001**, *103*, 449-457.
61. Soldati, R.; Berger, E.; Zenclussen, A.C.; Jorch, G.; Lode, H.N.; Salatino, M.; Rabinovich, G.A.; Fest, S. Neuroblastoma triggers an immunoevasive program involving galectin-1-dependent modulation of T cell and dendritic cell compartments. *Int J Cancer* **2012**, *131*, 1131-1141.
62. Kloss, C.C.; Lee, J.; Zhang, A.; Chen, F.; Melenhorst, J.J.; Lacey, S.F.; Maus, M.V.; Fraietta, J.A.; Zhao, Y.; June, C.H. Dominant-Negative TGF-beta Receptor Enhances PSMA-Targeted Human CAR T Cell Proliferation And Augments Prostate Cancer Eradication. *Mol Ther* **2018**, *26*, 1855-1866.
63. Vanichapol, T.; Chiangjong, W.; Panachan, J.; Anurathapan, U.; Chutipongtanate, S.; Hongeng, S. Secretory High-Mobility Group Box 1 Protein Affects Regulatory T Cell Differentiation in Neuroblastoma Microenvironment In Vitro. *J Oncol* **2018**, *2018*, 7946021.
64. Hainaut, P.; Plymoth, A. Targeting the hallmarks of cancer: towards a rational approach to next-generation cancer therapy. *Curr Opin Oncol* **2013**, *25*, 50-51.
65. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: the next generation. *Cell* **2011**, *144*, 646-674.
66. Nagarsheth, N.; Wicha, M.S.; Zou, W.P. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol* **2017**, *17*, 559-572.
67. Craddock, J.A.; Lu, A.; Bear, A.; Pule, M.; Brenner, M.K.; Rooney, C.M.; Foster, A.E. Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. *J Immunother* **2010**, *33*, 780-788.
68. Di Stasi, A.; De Angelis, B.; Rooney, C.M.; Zhang, L.; Mahendravada, A.; Foster, A.E.; Heslop, H.E.; Brenner, M.K.; Dotti, G.; Savoldo, B. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. *Blood* **2009**, *113*, 6392-6402.
69. Mohammed, S.; Sukumaran, S.; Bajgain, P.; Watanabe, N.; Heslop, H.E.; Rooney, C.M.; Brenner, M.K.; Fisher, W.E.; Leen, A.M.; Vera, J.F. Improving Chimeric Antigen Receptor-Modified T Cell Function by Reversing the Immunosuppressive Tumor Microenvironment of Pancreatic Cancer. *Mol Ther* **2017**, *25*, 249-258.
70. Davila, M.L.; Papapetrou, E.P. CARs Move To the Fast Lane. *Mol Ther* **2014**, *22*, 477-478.
71. Prasongtanakij, S.; Anurathapan, U.; Vanichapol, T.; Jittorntrum, B.; Atjanasuppat, K.; Pongpitcha, P.; Pakakasama, S.; Songdej, D.; Sirachainan, N.; Paisooksantivatana, K., et al. Production and characterization of haploidentical CD19 CAR T cells: Validated to induce a continuous complete remission in a patient with relapsed refractory B-cell ALL. *Asia Pac J Clin Oncol* **2020**, 10.1111/ajco.13474.

72. Feins, S.; Kong, W.; Williams, E.F.; Milone, M.C.; Fraietta, J.A. An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer. *Am J Hematol* **2019**, *94*, S3-S9.
73. Shusterman, S.; London, W.B.; Gillies, S.D.; Hank, J.A.; Voss, S.D.; Seeger, R.C.; Reynolds, C.P.; Kimball, J.; Albertini, M.R.; Wagner, B., et al. Antitumor activity of hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study. *J Clin Oncol* **2010**, *28*, 4969-4975.
74. Louis, C.U.; Savoldo, B.; Dotti, G.; Pule, M.; Yvon, E.; Myers, G.D.; Rossig, C.; Russell, H.V.; Diouf, O.; Liu, E., et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood* **2011**, *118*, 6050-6056.
75. Abramson, J.S.; McGree, B.; Noyes, S.; Plummer, S.; Wong, C.; Chen, Y.B.; Palmer, E.; Albertson, T.; Ferry, J.A.; Arrillaga-Romany, I.C. Anti-CD19 CAR T Cells in CNS Diffuse Large-B-Cell Lymphoma. *N Engl J Med* **2017**, *377*, 783-784.
76. Heczey, A.; Liu, D.; Tian, G.; Courtney, A.N.; Wei, J.; Marinova, E.; Gao, X.; Guo, L.; Yvon, E.; Hicks, J., et al. Invariant NKT cells with chimeric antigen receptor provide a novel platform for safe and effective cancer immunotherapy. *Blood* **2014**, *124*, 2824-2833.
77. Harrer, D.C.; Simon, B.; Fujii, S.I.; Shimizu, K.; Uslu, U.; Schuler, G.; Gerer, K.F.; Hoyer, S.; Dorrie, J.; Schaft, N. RNA-transfection of gamma/delta T cells with a chimeric antigen receptor or an alpha/beta T-cell receptor: a safer alternative to genetically engineered alpha/beta T cells for the immunotherapy of melanoma. *BMC Cancer* **2017**, *17*, 551.
78. Daher, M.; Rezvani, K. Next generation natural killer cells for cancer immunotherapy: the promise of genetic engineering. *Curr Opin Immunol* **2018**, *51*, 146-153.
79. Turtle, C.J.; Hanafi, L.A.; Berger, C.; Hudecek, M.; Pender, B.; Robinson, E.; Hawkins, R.; Chaney, C.; Cherian, S.; Chen, X., et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. *Sci Transl Med* **2016**, *8*, 355ra116.
80. Shah, N.N.; Fry, T.J. Mechanisms of resistance to CAR T cell therapy. *Nat Rev Clin Oncol* **2019**, *16*, 372-385.
81. Schmueck-Henneresse, M.; Omer, B.; Shum, T.; Tashiro, H.; Mamonkin, M.; Lapteva, N.; Sharma, S.; Rollins, L.; Dotti, G.; Reinke, P., et al. Comprehensive Approach for Identifying the T Cell Subset Origin of CD3 and CD28 Antibody-Activated Chimeric Antigen Receptor-Modified T Cells. *J Immunol* **2017**, *199*, 348-362.
82. Graef, P.; Buchholz, V.R.; Stemberger, C.; Flossdorf, M.; Henkel, L.; Schiemann, M.; Drexler, I.; Hofer, T.; Riddell, S.R.; Busch, D.H. Serial transfer of single-cell-derived immunocompetence reveals stemness of CD8(+) central memory T cells. *Immunity* **2014**, *41*, 116-126.
83. Tian, G.; Courtney, A.N.; Jena, B.; Heczey, A.; Liu, D.; Marinova, E.; Guo, L.; Xu, X.; Torikai, H.; Mo, Q., et al. CD62L+ NKT cells have prolonged persistence and antitumor activity in vivo. *J Clin Invest* **2016**, *126*, 2341-2355.
84. Sommermeyer, D.; Hudecek, M.; Kosasih, P.L.; Gogishvili, T.; Maloney, D.G.; Turtle, C.J.; Riddell, S.R. Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo. *Leukemia* **2016**, *30*, 492-500.
85. Rodriguez-Garcia, A.; Palazon, A.; Noguera-Ortega, E.; Powell, D.J., Jr.; Guedan, S. CAR-T Cells Hit the Tumor Microenvironment: Strategies to Overcome Tumor Escape. *Front Immunol* **2020**, *11*, 1109.
86. Jamali, A.; Hadjati, J.; Madjd, Z.; Mirzaei, H.R.; Thalheimer, F.B.; Agarwal, S.; Bonig, H.; Ullrich, E.; Hartmann, J. Highly Efficient Generation of Transgenically Augmented CAR NK Cells Overexpressing CXCR4. *Front Immunol* **2020**, *11*, 2028.
87. Tseng, H.C.; Xiong, W.; Badeti, S.; Yang, Y.; Ma, M.; Liu, T.; Ramos, C.A.; Dotti, G.; Fritzky, L.; Jiang, J.G., et al. Efficacy of anti-CD147 chimeric antigen receptors targeting hepatocellular carcinoma. *Nat Commun* **2020**, *11*, 4810.
88. Morgan, M.A.; Buning, H.; Sauer, M.; Schambach, A. Use of Cell and Genome Modification Technologies to Generate Improved "Off-the-Shelf" CAR T and CAR NK Cells. *Front Immunol* **2020**, *11*, 1965.
89. Caligiuri, M.A. Human natural killer cells. *Blood* **2008**, *112*, 461-469.
90. Parihar, R.; Rivas, C.; Huynh, M.; Omer, B.; Lapteva, N.; Metelitsa, L.S.; Gottschalk, S.M.; Rooney, C.M. NK Cells Expressing a Chimeric Activating Receptor Eliminate MDSCs and Rescue Impaired CAR-T Cell Activity against Solid Tumors. *Cancer Immunol Res* **2019**, *7*, 363-375.
91. Dasgupta, S.; Bhattacharya-Chatterjee, M.; O'Malley, B.W., Jr.; Chatterjee, S.K. Inhibition of NK cell activity through TGF-beta 1 by down-regulation of NKG2D in a murine model of head and neck cancer. *J Immunol* **2005**, *175*, 5541-5550.
92. Porcelli, S.; Yockey, C.E.; Brenner, M.B.; Balk, S.P. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. *J Exp Med* **1993**, *178*, 1-16.
93. Bendelac, A. CD1: presenting unusual antigens to unusual T lymphocytes. *Science* **1995**, *269*, 185-186.

94. Metelitsa, L.S. Anti-tumor potential of type-I NKT cells against CD1d-positive and CD1d-negative tumors in humans. *Clin Immunol* **2011**, *140*, 119-129.
95. Vivier, E.; Ugolini, S.; Blaise, D.; Chabannon, C.; Brossay, L. Targeting natural killer cells and natural killer T cells in cancer. *Nat Rev Immunol* **2012**, *12*, 239-252.
96. Olle Hurtado, M.; Wolbert, J.; Fisher, J.; Flutter, B.; Stafford, S.; Barton, J.; Jain, N.; Barone, G.; Majani, Y.; Anderson, J. Tumor infiltrating lymphocytes expanded from pediatric neuroblastoma display heterogeneity of phenotype and function. *PLoS One* **2019**, *14*, e0216373.
97. Xu, X.; Huang, W.; Heczey, A.; Liu, D.; Guo, L.; Wood, M.; Jin, J.; Courtney, A.N.; Liu, B.; Di Pierro, E.J., et al. NKT Cells Coexpressing a GD2-Specific Chimeric Antigen Receptor and IL15 Show Enhanced In Vivo Persistence and Antitumor Activity against Neuroblastoma. *Clin Cancer Res* **2019**, *25*, 7126-7138.
98. Fisher, J.; Kramer, A.M.; Gustafsson, K.; Anderson, J. Non-V delta 2 gamma delta T lymphocytes as effectors of cancer immunotherapy. *Oncoimmunology* **2015**, *4*, e973808.
99. Fisher, J.P.; Heuijerjans, J.; Yan, M.; Gustafsson, K.; Anderson, J. gammadelta T cells for cancer immunotherapy: A systematic review of clinical trials. *Oncoimmunology* **2014**, *3*, e27572.
100. Himoudi, N.; Morgenstern, D.A.; Yan, M.; Vernay, B.; Saraiva, L.; Wu, Y.; Cohen, C.J.; Gustafsson, K.; Anderson, J. Human gammadelta T lymphocytes are licensed for professional antigen presentation by interaction with opsonized target cells. *J Immunol* **2012**, *188*, 1708-1716.
101. Di Lorenzo, B.; Ravens, S.; Silva-Santos, B. High-throughput analysis of the human thymic Vdelta1(+) T cell receptor repertoire. *Sci Data* **2019**, *6*, 115.
102. Brandes, M.; Willimann, K.; Moser, B. Professional antigen-presentation function by human gammadelta T Cells. *Science* **2005**, *309*, 264-268.
103. Fisher, J.P.; Yan, M.; Heuijerjans, J.; Carter, L.; Abolhassani, A.; Frosch, J.; Wallace, R.; Flutter, B.; Capsomidis, A.; Hubank, M., et al. Neuroblastoma killing properties of Vdelta2 and Vdelta2-negative gammadeltaT cells following expansion by artificial antigen-presenting cells. *Clin Cancer Res* **2014**, *20*, 5720-5732.
104. Hamid, O.; Carvajal, R.D. Anti-programmed death-1 and anti-programmed death-ligand 1 antibodies in cancer therapy. *Expert Opin Biol Ther* **2013**, *13*, 847-861.
105. Sadelain, M.; Brentjens, R.; Riviere, I. The basic principles of chimeric antigen receptor design. *Cancer Discov* **2013**, *3*, 388-398.
106. Jensen, M.C.; Riddell, S.R. Designing chimeric antigen receptors to effectively and safely target tumors. *Curr Opin Immunol* **2015**, *33*, 9-15.
107. Alabanza, L.; Pegues, M.; Geldres, C.; Shi, V.; Wiltzius, J.J.W.; Sievers, S.A.; Yang, S.; Kochenderfer, J.N. Function of Novel Anti-CD19 Chimeric Antigen Receptors with Human Variable Regions Is Affected by Hinge and Transmembrane Domains. *Mol Ther* **2017**, *25*, 2452-2465.
108. Rafiq, S.; Hackett, C.S.; Brentjens, R.J. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat Rev Clin Oncol* **2020**, *17*, 147-167.
109. Suck, G.; Odendahl, M.; Nowakowska, P.; Seidl, C.; Wels, W.S.; Klingemann, H.G.; Tonn, T. NK-92: an 'off-the-shelf therapeutic' for adoptive natural killer cell-based cancer immunotherapy. *Cancer Immunol Immunother* **2016**, *65*, 485-492.
110. Bryceson, Y.T.; Chiang, S.C.; Darmanin, S.; Fauriat, C.; Schlums, H.; Theorell, J.; Wood, S.M. Molecular mechanisms of natural killer cell activation. *J Innate Immun* **2011**, *3*, 216-226.
111. Salter, A.I.; Pont, M.J.; Riddell, S.R. Chimeric antigen receptor-modified T cells: CD19 and the road beyond. *Blood* **2018**, *131*, 2621-2629.
112. Rozenbaum, M.; Meir, A.; Aharony, Y.; Itzhaki, O.; Schachter, J.; Bank, I.; Jacoby, E.; Besser, M.J. Gamma-Delta CAR-T Cells Show CAR-Directed and Independent Activity Against Leukemia. *Front Immunol* **2020**, *11*, 1347.
113. Lo Presti, E.; Pizzolato, G.; Corsale, A.M.; Caccamo, N.; Sireci, G.; Dieli, F.; Meraviglia, S. gammadelta T Cells and Tumor Microenvironment: From Immunosurveillance to Tumor Evasion. *Front Immunol* **2018**, *9*, 1395.
114. Mirzaei, H.R.; Mirzaei, H.; Lee, S.Y.; Hadjati, J.; Till, B.G. Prospects for chimeric antigen receptor (CAR) gammadelta T cells: A potential game changer for adoptive T cell cancer immunotherapy. *Cancer Lett* **2016**, *380*, 413-423.
115. Oelsner, S.; Friede, M.E.; Zhang, C.; Wagner, J.; Badura, S.; Bader, P.; Ullrich, E.; Ottmann, O.G.; Klingemann, H.; Tonn, T., et al. Continuously expanding CAR NK-92 cells display selective cytotoxicity against B-cell leukemia and lymphoma. *Cytotherapy* **2017**, *19*, 235-249.
116. Huang, Y.; Zeng, J.; Liu, T.; Xu, Q.; Song, X.; Zeng, J. DNAM1 and 2B4 Costimulatory Domains Enhance the Cytotoxicity of Anti-GPC3 Chimeric Antigen Receptor-Modified Natural Killer Cells Against Hepatocellular Cancer Cells in vitro. *Cancer Manag Res* **2020**, *12*, 3247-3255.

117. Fernandez, L.; Metais, J.Y.; Escudero, A.; Vela, M.; Valentin, J.; Vallcorba, I.; Leivas, A.; Torres, J.; Valeri, A.; Patino-Garcia, A., et al. Memory T Cells Expressing an NKG2D-CAR Efficiently Target Osteosarcoma Cells. *Clin Cancer Res* **2017**, *23*, 5824-5835.
118. Ng, Y.Y.; Tay, J.C.K.; Li, Z.; Wang, J.; Zhu, J.; Wang, S. T Cells Expressing NKG2D CAR with a DAP12 Signaling Domain Stimulate Lower Cytokine Production While Effective in Tumor Eradication. *Mol Ther* **2020**, 10.1016/j.ymthe.2020.08.016.
119. Tan, A.H.J.; Vinanica, N.; Campana, D. Chimeric antigen receptor-T cells with cytokine neutralizing capacity. *Blood Adv* **2020**, *4*, 1419-1431.
120. Moretta, A.; Biassoni, R.; Bottino, C.; Pende, D.; Vitale, M.; Poggi, A.; Mingari, M.C.; Moretta, L. Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. *Immunol Rev* **1997**, *155*, 105-117.
121. Wang, E.; Wang, L.C.; Tsai, C.Y.; Bhoj, V.; Gershenson, Z.; Moon, E.; Newick, K.; Sun, J.; Lo, A.; Baradet, T., et al. Generation of Potent T-cell Immunotherapy for Cancer Using DAP12-Based, Multichain, Chimeric Immunoreceptors. *Cancer Immunol Res* **2015**, *3*, 815-826.
122. Topfer, K.; Cartellieri, M.; Michen, S.; Wiedemuth, R.; Muller, N.; Lindemann, D.; Bachmann, M.; Fussel, M.; Schackert, G.; Temme, A. DAP12-based activating chimeric antigen receptor for NK cell tumor immunotherapy. *J Immunol* **2015**, *194*, 3201-3212.
123. Xu, X.; Zhao, W.; Yue, Z.; Qin, M.; Jin, M.; Chang, L.J.; Ma, X. 4SCAR-GD2-modified T-cell therapy in neuroblastoma with MYCN amplification: A case report with over 4-year follow-up data. *Pediatr Investig* **2020**, *4*, 55-58.
124. Vanichapol, T.; Chutipongtanate, S.; Anurathapan, U.; Hongeng, S. Immune Escape Mechanisms and Future Prospects for Immunotherapy in Neuroblastoma. *Biomed Res Int* **2018**, *2018*, 1812535.
125. Trujillo, J.A.; Sweis, R.F.; Bao, R.; Luke, J.J. T Cell-Inflamed versus Non-T Cell-Inflamed Tumors: A Conceptual Framework for Cancer Immunotherapy Drug Development and Combination Therapy Selection. *Cancer Immunol Res* **2018**, *6*, 990-1000.
126. Kershaw, M.H.; Westwood, J.A.; Parker, L.L.; Wang, G.; Eshhar, Z.; Mavroukakis, S.A.; White, D.E.; Wunderlich, J.R.; Canevari, S.; Rogers-Freezer, L., et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res* **2006**, *12*, 6106-6115.
127. Liu, G.; Rui, W.; Zheng, H.; Huang, D.; Yu, F.; Zhang, Y.; Dong, J.; Zhao, X.; Lin, X. CXCR2-modified CAR-T cells have enhanced trafficking ability that improves treatment of hepatocellular carcinoma. *Eur J Immunol* **2020**, *50*, 712-724.
128. Hughes, C.E.; Nibbs, R.J.B. A guide to chemokines and their receptors. *FEBS J* **2018**, *285*, 2944-2971.
129. Curiel, T.J.; Coukos, G.; Zou, L.; Alvarez, X.; Cheng, P.; Mottram, P.; Evdemon-Hogan, M.; Conejo-Garcia, J.R.; Zhang, L.; Burow, M., et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* **2004**, *10*, 942-949.
130. Dimberg, A. Chemokines in angiogenesis. *Curr Top Microbiol Immunol* **2010**, *341*, 59-80.
131. Shields, J.D.; Kourtis, I.C.; Tomei, A.A.; Roberts, J.M.; Swartz, M.A. Induction of lymphoidlike stroma and immune escape by tumors that express the chemokine CCL21. *Science* **2010**, *328*, 749-752.
132. Pivarcsi, A.; Muller, A.; Hippe, A.; Rieker, J.; van Lierop, A.; Steinhoff, M.; Seeliger, S.; Kubitza, R.; Pippirs, U.; Meller, S., et al. Tumor immune escape by the loss of homeostatic chemokine expression. *Proc Natl Acad Sci U S A* **2007**, *104*, 19055-19060.
133. Zlotnik, A.; Burkhardt, A.M.; Homey, B. Homeostatic chemokine receptors and organ-specific metastasis. *Nat Rev Immunol* **2011**, *11*, 597-606.
134. Stone, M.J.; Hayward, J.A.; Huang, C.; Z, E.H.; Sanchez, J. Mechanisms of Regulation of the Chemokine-Receptor Network. *Int J Mol Sci* **2017**, *18*.
135. Whilding, L.M.; Halim, L.; Draper, B.; Parente-Pereira, A.C.; Zabinski, T.; Davies, D.M.; Maher, J. CAR T-Cells Targeting the Integrin alphavbeta6 and Co-Expressing the Chemokine Receptor CXCR2 Demonstrate Enhanced Homing and Efficacy against Several Solid Malignancies. *Cancers (Basel)* **2019**, *11*.
136. Adachi, K.; Kano, Y.; Nagai, T.; Okuyama, N.; Sakoda, Y.; Tamada, K. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat Biotechnol* **2018**, *36*, 346-351.
137. Liu, L.B.; Xie, F.; Chang, K.K.; Li, M.Q.; Meng, Y.H.; Wang, X.H.; Li, H.; Li, D.J.; Yu, J.J. Hypoxia promotes the proliferation of cervical carcinoma cells through stimulating the secretion of IL-8. *Int J Clin Exp Pathol* **2014**, *7*, 575-583.
138. Kim, E.S.; Serur, A.; Huang, J.; Manley, C.A.; McCrudden, K.W.; Frischer, J.S.; Soffer, S.Z.; Ring, L.; New, T.; Zabski, S., et al. Potent VEGF blockade causes regression of coopted vessels in a model of neuroblastoma. *Proc Natl Acad Sci U S A* **2002**, *99*, 11399-11404.

139. Ara, T.; Song, L.; Shimada, H.; Keshelava, N.; Russell, H.V.; Metelitsa, L.S.; Groshen, S.G.; Seeger, R.C.; DeClerck, Y.A. Interleukin-6 in the bone marrow microenvironment promotes the growth and survival of neuroblastoma cells. *Cancer Res* **2009**, *69*, 329-337.
140. Brignole, C.; Marimpietri, D.; Pastorino, F.; Di Paolo, D.; Pagnan, G.; Loi, M.; Piccardi, F.; Cilli, M.; Tradori-Cappai, A.; Arrigoni, G., et al. Anti-IL-10R antibody improves the therapeutic efficacy of targeted liposomal oligonucleotides. *J Control Release* **2009**, *138*, 122-127.
141. Gabrilovich, D.I.; Nagaraj, S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* **2009**, *9*, 162-174.
142. Marvel, D.; Gabrilovich, D.I. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. *J Clin Invest* **2015**, *125*, 3356-3364.
143. Diaz-Montero, C.M.; Salem, M.L.; Nishimura, M.I.; Garrett-Mayer, E.; Cole, D.J.; Montero, A.J. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol Immunother* **2009**, *58*, 49-59.
144. Noguchi, T.; Ward, J.P.; Gubin, M.M.; Arthur, C.D.; Lee, S.H.; Hundal, J.; Selby, M.J.; Graziano, R.F.; Mardis, E.R.; Korman, A.J., et al. Temporally Distinct PD-L1 Expression by Tumor and Host Cells Contributes to Immune Escape. *Cancer Immunol Res* **2017**, *5*, 106-117.
145. Whittle, S.B.; Smith, V.; Doherty, E.; Zhao, S.; McCarty, S.; Zage, P.E. Overview and recent advances in the treatment of neuroblastoma. *Expert Rev Anticancer Ther* **2017**, *17*, 369-386.
146. Pinto, N.R.; Applebaum, M.A.; Volchenboum, S.L.; Matthay, K.K.; London, W.B.; Ambros, P.F.; Nakagawara, A.; Berthold, F.; Schleiermacher, G.; Park, J.R., et al. Advances in Risk Classification and Treatment Strategies for Neuroblastoma. *J Clin Oncol* **2015**, *33*, 3008-3017.
147. Hui, E.; Cheung, J.; Zhu, J.; Su, X.; Taylor, M.J.; Wallweber, H.A.; Sasmal, D.K.; Huang, J.; Kim, J.M.; Mellman, I., et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science* **2017**, *355*, 1428-1433.
148. Thery, C.; Witwer, K.W.; Aikawa, E.; Alcaraz, M.J.; Anderson, J.D.; Andriantsitohaina, R.; Antoniou, A.; Arab, T.; Archer, F.; Atkin-Smith, G.K., et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* **2018**, *7*, 1535750.
149. Antonyak, M.A.; Cerione, R.A. Microvesicles as mediators of intercellular communication in cancer. *Methods Mol Biol* **2014**, *1165*, 147-173.
150. Blavier, L.; Yang, R.M.; DeClerck, Y.A. The Tumor Microenvironment in Neuroblastoma: New Players, New Mechanisms of Interaction and New Perspectives. *Cancers (Basel)* **2020**, *12*.
151. Becker, A.; Thakur, B.K.; Weiss, J.M.; Kim, H.S.; Peinado, H.; Lyden, D. Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. *Cancer Cell* **2016**, *30*, 836-848.
152. Fonseka, P.; Liem, M.; Ozcitti, C.; Adda, C.G.; Ang, C.S.; Mathivanan, S. Exosomes from N-Myc amplified neuroblastoma cells induce migration and confer chemoresistance to non-N-Myc amplified cells: implications of intra-tumour heterogeneity. *J Extracell Vesicles* **2019**, *8*, 1597614.
153. Wortzel, I.; Dror, S.; Kenific, C.M.; Lyden, D. Exosome-Mediated Metastasis: Communication from a Distance. *Dev Cell* **2019**, *49*, 347-360.
154. Ali, S.; Toews, K.; Schwiebert, S.; Klaus, A.; Winkler, A.; Grunewald, L.; Oevermann, L.; Deubzer, H.E.; Tuns, A.; Jensen, M.C., et al. Tumor-Derived Extracellular Vesicles Impair CD171-Specific CD4(+) CAR T Cell Efficacy. *Front Immunol* **2020**, *11*, 531.
155. Peinado, H.; Aleckovic, M.; Lavotshkin, S.; Matei, I.; Costa-Silva, B.; Moreno-Bueno, G.; Hergueta-Redondo, M.; Williams, C.; Garcia-Santos, G.; Ghajar, C., et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med* **2012**, *18*, 883-891.
156. Costa-Silva, B.; Aiello, N.M.; Ocean, A.J.; Singh, S.; Zhang, H.; Thakur, B.K.; Becker, A.; Hoshino, A.; Mark, M.T.; Molina, H., et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol* **2015**, *17*, 816-826.
157. Ji, Q.; Zhou, L.; Sui, H.; Yang, L.; Wu, X.; Song, Q.; Jia, R.; Li, R.; Sun, J.; Wang, Z., et al. Primary tumors release ITGBL1-rich extracellular vesicles to promote distal metastatic tumor growth through fibroblast-niche formation. *Nat Commun* **2020**, *11*, 1211.
158. Nakata, R.; Shimada, H.; Fernandez, G.E.; Fanter, R.; Fabbri, M.; Malvar, J.; Zimmermann, P.; DeClerck, Y.A. Contribution of neuroblastoma-derived exosomes to the production of pro-tumorigenic signals by bone marrow mesenchymal stromal cells. *J Extracell Vesicles* **2017**, *6*, 1332941.
159. Challagundla, K.B.; Wise, P.M.; Neviani, P.; Chava, H.; Murtadha, M.; Xu, T.; Kennedy, R.; Ivan, C.; Zhang, X.; Vannini, I., et al. Exosome-mediated transfer of microRNAs within the tumor microenvironment and neuroblastoma resistance to chemotherapy. *J Natl Cancer Inst* **2015**, *107*.

160. Keir, M.E.; Butte, M.J.; Freeman, G.J.; Sharpe, A.H. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* **2008**, *26*, 677-704.
161. Yokosuka, T.; Takamatsu, M.; Kobayashi-Imanishi, W.; Hashimoto-Tane, A.; Azuma, M.; Saito, T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *J Exp Med* **2012**, *209*, 1201-1217.
162. Gargett, T.; Yu, W.; Dotti, G.; Yvon, E.S.; Christo, S.N.; Hayball, J.D.; Lewis, I.D.; Brenner, M.K.; Brown, M.P. GD2-specific CAR T Cells Undergo Potent Activation and Deletion Following Antigen Encounter but can be Protected From Activation-induced Cell Death by PD-1 Blockade. *Mol Ther* **2016**, *24*, 1135-1149.
163. Toews, K.; Grunewald, L.; Schwiebert, S.; Klaus, A.; Winkler, A.; Ali, S.; Zirngibl, F.; Astrahantseff, K.; Wagner, D.L.; Henssen, A.G., et al. Central memory phenotype drives success of checkpoint inhibition in combination with CAR T cells. *Mol Carcinog* **2020**, *59*, 724-735.
164. Jabbari, P.; Hanaei, S.; Rezaei, N. State of the art in immunotherapy of neuroblastoma. *Immunotherapy* **2019**, *11*, 831-850.
165. Sanmamed, M.F.; Carranza-Rua, O.; Alfaro, C.; Onate, C.; Martin-Algarra, S.; Perez, G.; Landazuri, S.F.; Gonzalez, A.; Gross, S.; Rodriguez, I., et al. Serum interleukin-8 reflects tumor burden and treatment response across malignancies of multiple tissue origins. *Clin Cancer Res* **2014**, *20*, 5697-5707.
166. Kuwada, Y.; Sasaki, T.; Morinaka, K.; Kitadai, Y.; Mukaida, N.; Chayama, K. Potential involvement of IL-8 and its receptors in the invasiveness of pancreatic cancer cells. *Int J Oncol* **2003**, *22*, 765-771.
167. Chen, Y.; Sun, C.; Landoni, E.; Metelitsa, L.; Dotti, G.; Savoldo, B. Eradication of Neuroblastoma by T Cells Redirected with an Optimized GD2-Specific Chimeric Antigen Receptor and Interleukin-15. *Clin Cancer Res* **2019**, *25*, 2915-2924.
168. Alvarez-Rueda, N.; Desselle, A.; Cochonneau, D.; Chaumette, T.; Clemenceau, B.; Leprieur, S.; Bougras, G.; Supiot, S.; Mussini, J.M.; Barbet, J., et al. A monoclonal antibody to O-acetyl-GD2 ganglioside and not to GD2 shows potent anti-tumor activity without peripheral nervous system cross-reactivity. *PLoS One* **2011**, *6*, e25220.
169. Kramer, K.; Khan, O.; Haque, S. Central Nervous System Neuroblastoma Metastases Pseudoprogression Following Intraventricular Anti-B7h3 Radioimmunotherapy. *Neuro-Oncology* **2019**, *21*, 95-95.
170. Yang, L.H.; Ma, X.L.; Liu, Y.C.; Zhao, W.; Yu, L.H.; Qin, M.Q.; Zhu, G.H.; Wang, K.; Shi, X.D.; Zhang, Z.X., et al. Chimeric Antigen Receptor 4SCAR-GD2-Modified T Cells Targeting High-Risk and Recurrent Neuroblastoma: A Phase II Multi-Center Trial in China. *Blood* **2017**, *130*.
171. Zhao, W.; Dong, L.; Wang, H.; Zheng, X.; Zhang, C.; Wang, K.; Zhu, G.; Wang, B.; Qin, M.; Liu, Y., et al. Chimeric Antigen Receptor 4SCAR-GD2/CD56-Modified T Cells in the Refractory and Recurrent Paediatric Solid Tumors. *Pediatric Blood & Cancer* **2016**, *63*, S38-S38.