

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Review

The immune landscape of neuroblastoma: Challenges and opportunities for novel therapeutic strategies in pediatric oncology



Judith Wienke ^{a,*}, Miranda P. Dierselhuis ^a, Godelieve A.M. Tytgat ^a,
Annette Künkele ^b, Stefan Nierkens ^a, Jan J. Molenaar ^a

^a Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands

^b Department of Pediatric Oncology and Hematology, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt - Universität zu Berlin, Berlin Institute of Health, Berlin, Germany

Received 26 October 2020; accepted 3 November 2020

Available online 18 December 2020

KEYWORDS

Neuroblastoma;
Tumour immunology;
Immunotherapy;
Tumour
microenvironment;
T cells;
NK cells;
Tumour immune
evasion

Abstract Immunotherapy holds great promise for the treatment of pediatric cancers. In neuroblastoma, the recent implementation of anti-GD2 antibody Dinutuximab into the standard of care has improved patient outcomes substantially. However, 5-year survival rates are still below 50% in patients with high-risk neuroblastoma, which has sparked investigations into novel immunotherapeutic approaches. T cell-engaging therapies such as immune checkpoint blockade, antibody-mediated therapy and adoptive T cell therapy have proven remarkably successful in a range of adult cancers but still meet challenges in pediatric oncology. In neuroblastoma, their limited success may be due to several factors. Neuroblastoma displays low immunogenicity due to its low mutational load and lack of MHC-I expression. Tumour infiltration by T and NK cells is especially low in high-risk neuroblastoma and is prognostic for survival. Only a small fraction of tumour-infiltrating lymphocytes shows tumour reactivity. Moreover, neuroblastoma tumours employ a variety of immune evasion strategies, including expression of immune checkpoint molecules, induction of immunosuppressive myeloid and stromal cells, as well as secretion of immunoregulatory mediators, which reduce infiltration and reactivity of immune cells. Overcoming these challenges will be key to the successful implementation of novel immunotherapeutic interventions. Combining different immunotherapies, as well as personalised strategies, may be promising approaches. We will discuss the composition, function and prognostic value of tumour-infiltrating lymphocytes (TIL) in neuroblastoma, reflect on challenges for immunotherapy, including a lack of TIL reactivity and tumour immune evasion strategies, and highlight opportunities for immunotherapy and future

* Corresponding author: Princess Máxima Center for Pediatric Oncology, Heidelberglaan 25, 3584 CS, Utrecht, the Netherlands.
E-mail address: j.wienke-4@prinsesmaximacentrum.nl (J. Wienke).

perspectives with regard to state-of-the-art developments in the tumour immunology space.
© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Immunotherapy has recently led to a leap in survival rates in a variety of adult cancers [1]. State of the art immune interventions include immune checkpoint inhibition (ICI), antibody-mediated therapy and adoptive T cell therapy. Although immunotherapy may also hold great promise for pediatric oncology, it is still in the early stages of development for pediatric solid cancers. The increased survival of patients with high-risk neuroblastoma following the implementation of anti-GD2 therapy into standard care exemplifies the potential of immunotherapy in pediatric oncology [2].

Neuroblastoma is one of the most common extracranial solid tumours in children, arising from neural crest progenitor cells in the sympathetic nervous system. It presents with tumour masses in the adrenal glands and/or sympathetic ganglia. The prognosis of neuroblastoma depends heavily on the disease stage [3,4]. Patients are stratified into risk groups based on molecular factors and disease presentation, including patient age, tumour histology, localised versus metastatic disease, and genomic alterations, among which *MYCN* amplification is an important driver of poor prognosis [4,5]. Whereas patients with low-risk (LR) disease have a favourable prognosis with >90% survival, the 5-year survival rate of patients with high-risk (HR) disease is still below 50% [3]. The current standard of care for patients with HR-neuroblastoma consists of an intense treatment protocol with induction chemotherapy, surgical tumour resection, consolidation with single or tandem high dose chemotherapy followed by autologous stem cell transplantation, radiotherapy and immunotherapy with the anti-GD2 antibody Dinutuximab (which is currently combined with isotretinoin and in some regions with GM-CSF, but not anymore with IL-2) [6]. Dinutuximab-dependent cytotoxicity is thought to be mainly mediated by neutrophils and natural killer (NK) cells [7,8]. The introduction of immunotherapy into the standard treatment regimen has significantly improved survival rates [6,9–13]. However, the current prognosis with less than 50% survival in patients with HR-neuroblastoma remains dismal, nonetheless, with disease relapses being an important factor.

The encouraging results of Dinutuximab, but still unsatisfactory prognosis, have sparked investigations into novel immunotherapeutic approaches, which may provide immunological memory against the tumour to prevent disease relapses. Immune interventions that have demonstrated great success in a range of adult

cancers, such as ICI and adoptive T cell therapy, however, meet challenges in pediatric oncology, resulting in limited effectivity [2,14]. First, pediatric solid cancers in general (and neuroblastoma in specific) have low immunogenicity, which can be attributed to a low mutational burden and consequently low neoepitope expression, as well as low expression of MHC-I [15–17]. This low immunogenicity results in a lack of tumour infiltration by lymphocytes and a suboptimal anti-tumour reactivity of the few tumour-infiltrating lymphocytes (TIL) present. Furthermore, tumour immune evasion strategies that are active both in the tumour microenvironment (TME) and systemically can additionally hamper lymphocyte infiltration and activity [18]. These factors, taken together, impede effective engagement of cytotoxic killer cells such as NK cells and cytotoxic T lymphocytes (CTL) during immunotherapy [1,2]. Successful implementation of novel immunotherapeutic interventions in pediatric oncology will, therefore, likely require a different or more extensive approach than in adults.

To provide a perspective for the development of novel immunotherapeutic strategies in neuroblastoma, we will 1) discuss the composition, function and prognostic value of TIL in neuroblastoma, 2) reflect on challenges for immunotherapy, including a lack of TIL reactivity and tumour immune evasion strategies, and 3) highlight opportunities for immunotherapy and future perspectives with regard to state-of-the-art developments in the tumour immunology space.

2. Tumour-infiltrating lymphocytes in neuroblastoma

2.1. TIL composition in human neuroblastoma

Although neuroblastoma has been considered to be an immunologically ‘cold’ tumour [19,20], multiple studies have demonstrated the presence of TIL, including T cells and NK cells, in human neuroblastoma tumours (Table 1 and Fig. 1) [21–42]. Also, invariant natural killer T cells (iNKT), NKT cells and $\gamma\delta$ T cells have been identified [34–36,43], whereas B cells are rare and mostly undetectable [21,22,41]. The number and composition of TIL vary significantly between individual patients and tumour samples, with TIL detected in 28%–100% of tumours by immunohistochemistry (IHC) [24–27]. CD3⁺ T cells make up ~5% of total cells in the tumour microenvironment (TME) [22,23], consist of both CD4⁺ and CD8⁺ T cells, and are more frequently detected in septa than tumour nests [28–30].

Table 1

TIL presence and distribution in human neuroblastoma. Disc = discovery cohort, FC = flow cytometry, ganglioNBL = ganglioneuroblastoma, HR = high-risk, IF = immunofluorescence, IHC = immunohistochemistry, IR = intermediate risk, LR = low-risk, MYCN-NA = MYCN non amplified, NBL = neuroblastoma, NHR = non high-risk, NK cells = natural killer cells, (i)NKT cells = (invariant) natural killer T cells, Pts = patients, (RT-q)PCR = (reverse transcriptase-quantitative) Polymerase Chain Reaction, seq = sequencing, TCR = T cell receptor, TIL = tumour infiltrating lymphocytes, Val = validation cohort, $\gamma\delta$ T = gammadelta T cells.

Ref	Patients/samples/ material	Stage	Method	Lymphocytes	T cells		Other lymphocytes	
					CD3	CD4, CD8 and Tregs	NK, NKT and $\gamma\delta$ T cells	B cells
[21]	9/9. Primary tumour	4	FC of ficoll-isolated cell fraction after digestion		10–80% (mean 50%) of total	CD4/CD8 ratio 0.5–5	NK: 1–30%	<2%
[22]	26/26. Primary tumour	3, 4	FC of total single-cell suspension after digestion		5% of viable cells			
[23]	8/8. Primary tumour versus blood	1, 2, 3, 4	FC of total single-cell suspension after digestion		0.4–17.8% (mean 4.5%) in TIL	CD4/CD8 ratio: TIL 0.3–1.7 (mean 0.8) versus PBL 0.2–2.7 (mean 1.5)		
[35]	24/30. Primary tumour	Unknown	FC of <i>ex vivo</i> expanded TIL		Present	CD4 and CD8 present	NKT present.	$\gamma\delta$ T present.
[24]	53/53. Primary tumour	1, 2, 3, 4	IHC	In 28% of pts				
[25]	23/23. Primary tumour	N/A	IHC	In 96% of pts				
[22]	26/26. Primary tumour	3, 4	IHC CD3, CD4 and CD8	Organised lymphoid tissue and B-cell follicles at edges of tumour nests	CD3, including CD4 and CD8: 88% of lymphocytes in peritumoural stroma	CD4 and CD8 minimal/ undetectable in nests	NK rare/ undetectable	rare/ undetectable
[33]	15/15. Primary tumour	LR, IR, HR	IHC CD45 and CD4	Present		CD4 present		
[27]	111 untreated and 8 treated. Primary tumour	1, 2, 3, 4/ LR, MR, HR	IHC H&E	In 83% of NBL and 89% of ganglioNBL				
[29]	3 HLA*A201 patients. NY-ESO-1+ tumours from metastatic relapse	4	IHC		Present in nests and septa			
[26]	21/21. Primary tumour	N/A	IHC CD3		in 100% of samples			
[30]	84/84. Primary tumour	1, 2, 3, 4	IHC CD3, CD4 and CD8		~1.5x more frequent in septa than nests	CD4 and CD8 ~2x more frequent in septa than nests		
[31]	55/55. Primary tumour and metastasis	HR, NHR	IHC CD8			CD8 present in ~50–100% of HR and NHR tumours		
[28]	36/36. Resection tumour		IHC CD8			CD8 present in septa, nests and perivascular		
[38]	80/80	1, 2, 3, 4, 4S	IHC CD8			CD8 present		
[36]	129/129 MYCN-NA. Primary tumour	4	IF				NKT present	
[40]	8/8. Primary tumour versus blood	2, 3, 4	PCR of TCR		Polyclonal TCR, diverse V α and V β . V β 2 clonality in tumours.			
[43]	98/98. Primary tumour	4	RT-qPCR V α 24-J α 18, Flow cytometry				iNKT in 53% of samples	

(continued on next page)

Table 1 (continued)

Ref	Patients/samples/material	Stage	Method	Lymphocytes	T cells		Other lymphocytes	
					CD3	CD4, CD8 and Tregs	NK, NKT and $\gamma\delta$ T cells	B cells
[34]	107/107. Primary tumour	1, 2, 3, 4, 4S	RT-qPCR $V\alpha 24$ - $J\alpha 18$				iNKT in 64% of samples	
[41]	2 patients (1 paired primary and resection, 1 resection)		10 \times single-cell RNA seq		~2% in primary, 10–40% of cells in resection		NK: Undetectable in primary, ~0–20% in resection	Undetectable in primary, ~5% of resection
[33]	TARGET (n = 148). Primary tumour	1, 2, 3, 4	Deconvolution of bulk RNA seq with CIBERSORT	Present	Present	CD4 and CD8 present	NK present	Present
[39]	498 primary NBL. Primary tumour	4	Deconvolution of bulk RNA seq with CIBERSORT. Immune signature from [243]			CD8 present. Tregs present.	NK present	
[38]	TARGET (n = 150). Val (n = 190). Primary tumour	3, 4, 4S	Deconvolution of bulk RNA seq with CIBERSORT, TCR seq		Present. Some TCR clonality in MYCN-NA	CD4 and CD8 present	NK present. $\gamma\delta$ T present.	Present
[37]	TARGET (n = 149). Val (n = 498), (n = 649), (n = 88). Primary tumour.	1, 2, 3, 4, 4S	Deconvolution of bulk RNA seq with xCell		Present	CD4 and CD8 present	NKT present	
[42]	TARGET (n = 160) and Val (n = 498). Primary tumour	1, 2, 3, 4, 4S	Deconvolution of bulk RNA seq with CIBERSORT		Present	CD4 and CD8 present	NK present	Present

Compared to blood, CD8⁺ T cells are preferentially present over CD4⁺ T cells in the TME, with reported

CD4/CD8 ratios ranging from 0.3 to 5 [21,23,29]. Functionally, TIL are able to produce IL-4, IL-5, IL-8,

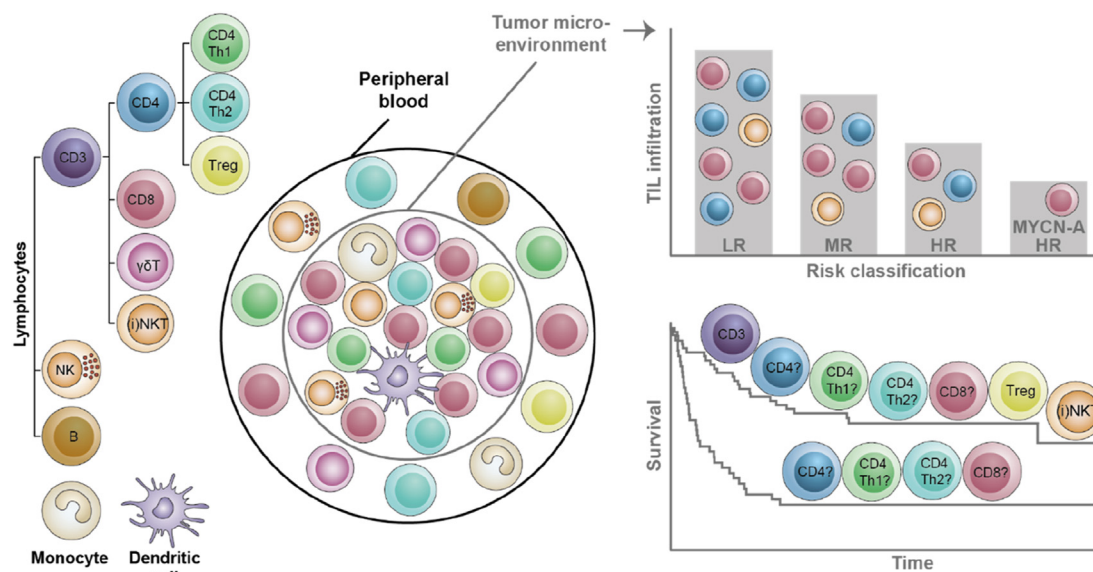


Fig. 1. **TIL composition and prognostic value.** T cells (CD8⁺ T cells more than CD4⁺ Th1, Th2 and Treg cells), NK cells, (i)NKT cells and a small number of B cells have been described in neuroblastoma tumours. High-risk (HR) tumours have less TIL infiltration than low-risk (LR) and medium risk (MR) tumours, with MYCN-A tumours having the lowest TIL infiltration. High infiltration of total CD3⁺ T cells, Tregs and (i)NKT cells has been associated with improved survival, while the prognostic value of CD4⁺ T cells, CD4⁺ T helper subsets and CD8⁺ T cells remains to be elucidated.

IL-10, interferon (IFN)- γ and TNF α mRNA in response to *ex vivo* stimulation with IL-2, which suggests a mixed population of both Th1 and Th2 cells [21]. The presence of Th17 cells in neuroblastoma has not been studied yet. NK cells were also variably detected, ranging from rare presence to constituting up to ~30% of TIL [21,22]. Since only two studies so far have compared *ex vivo* TIL characteristics to peripheral blood lymphocytes (PBL) from matched blood, one of which focused on the T cell receptor (TCR) repertoire, it is still unknown in which respect neuroblastoma-infiltrating lymphocytes have undergone functional specialisation and/or differentiation compared to their counterparts in blood.

Due to the limited availability of fresh neuroblastoma tumour material and the practical challenges of in-depth immune analyses, most studies describe a limited number of patients. Recent publications have resorted to computational methods to shed light on the immune composition of neuroblastoma and identified traces and signatures of immune infiltration by deconvolution of bulk RNA sequencing data of tumour samples [33,37–39]. At least 50% of cell components in the TME were predicted to consist of immune cells, half of which myeloid and half lymphoid [37]. The myeloid compartment was predicted to mainly consist of dendritic cells (DC), next to a small fraction of macrophages. The lymphoid compartment was dominated by CD4⁺ T helper cells, next to B cells and a small fraction of CD8⁺ cytotoxic T cells [37]. This contrasts with the CD8⁺ dominance proposed by IHC-based studies, which may be due to differences in definitions and sensitivity of the methods. NK cells, NKT cells and $\gamma\delta$ T cells were predicted to be present as well [33,37–39]. Of note, not only in primary but also in relapsed tumours, infiltration of CD3⁺ T cells has been demonstrated [29].

Recently, the first single-cell RNA sequencing study of neuroblastoma showed a remarkable increase of T cell infiltration after chemotherapy in a paired patient sample – one taken at diagnosis and one in resection material after neo-adjuvant chemotherapy – indicating that chemotherapy-induced immunogenic cell death may attract lymphocytes to the tumour. The amount of lymphocyte infiltration after chemotherapy differed substantially between tumours of different patients [41].

The discrepancies between IHC-based and computational studies highlight the need for more standardised, high-quality and in-depth immunophenotyping in neuroblastoma. Future studies using techniques with single-cell resolution, comparing TIL and patient-matched PBL, will be essential to gain more insight into TIL composition, functional specialisation and changes over time upon therapy or disease progression.

2.2. Prognostic value of TIL profile

2.2.1. Prognostic value of TIL abundance

Although it is still largely unclear whether differences in immune cell infiltration are due to tumour characteristics, medication effects, or other factors, the extent and type of TIL infiltration has been associated with prognosis (Fig. 1) [24,25]. Martin & Beckwith, 50 years ago, demonstrated a stepwise decrease of lymphocyte infiltration with increasing tumour stage and a higher 2-year survival in patients with infiltrates than without infiltrates (Survival: 64% in TIL^{high} versus 11% TIL^{low}) [24]. Lauder & Aherne reported a direct correlation between survival time and intensity of lymphocytic infiltration in the primary tumour ($r_s = 0.69$, $p < 0.001$) [25]. These findings are now supported by more recent studies, showing reduced CD3⁺, CD4⁺ and CD8⁺ T cell infiltration in high-risk, and especially in stage 4 tumours, as well as higher survival rates in patients with higher lymphocyte, CD3⁺ and CD4⁺ T cell infiltration (Table 2) [28,30,32,33,39,44]. Stratification of patients by CD3⁺ TIL density demonstrated a significantly higher disease-free survival (DFS), overall survival (OS) and event-free survival (EFS) in patients with a higher TIL density [30,32]. Although Martin & Beckwith did not find a prognostic value of TIL infiltration for survival rates within the group of stage 3/4 tumours (OS: 17% in TIL^{high} versus 11% in TIL^{low}) [24], two other studies did observe a survival benefit of TIL infiltration in stage 4 neuroblastoma [39,44]. Not only the amount but also the localisation and organisation of T cells in the TME has prognostic value. Neuroblastoma patients with favourable outcomes were characterised by a more structured T cell infiltrate, which was gradually lost in tumours with a poor prognosis. While proliferating T cells were localised in close proximity to tumour cells in stage 4S tumours, they were placed distant from tumour cells in stage 4 tumours. Moreover, stage 4S tumours were characterised by a higher number of proliferating Ki67⁺CD3⁺ T cells than stage 4 tumours [30]. The role of immune surveillance in the spontaneous regression of 4S tumours, however, remains to be elucidated [45].

Overall, these results suggest a strong relation between TIL density and both tumour stage and patient survival. One may speculate that the association between TIL infiltration and tumour stage is related to the differentiation grade of the tumour, since it is an important determinant of tumour risk stage, and may influence immunogenicity [46]. For example, the notoriously low MHC-I expression of neuroblastoma [16], rather than being the result of active downregulation, may reflect the pre-MHC-I expressing, undifferentiated state of the neural crest [47]. Moreover, *in vitro* differentiation of neuroblastoma cell lines and *ex vivo* tumours was associated with increased immunogenicity, e.g. upregulated MHC-I expression, and

Table 2

Prognostic significance of TIL presence and composition in human neuroblastoma. » = positively associated with, DC = dendritic cell, disc = discovery cohort, expr = expression, EFS = event-free survival, fav = favourable, FC = flow cytometry, GFEA = Gene functional enrichment analysis, HR = high-risk, IHC = immunohistochemistry, IR = intermediate risk, LR = low-risk, mono = monocytes, MYCN-(N) A = MYCN-(non) amplified, MØ = macrophage, NBL = neuroblastoma, NHR = non-high-risk, OS = overall survival, Pts = patients, (RT-q)PCR = (reverse transcriptase-quantitative) Polymerase Chain Reaction, seq = sequencing, Th = T helper, TIL = tumour infiltrating lymphocytes, unfav = unfavourable, val = validation cohort, WGCNA = Weighted Gene Coexpression Network Analysis.

Ref	Patients/ samples	Stage	Method	Tumour stage					Risk	Survival analysis		MYCN
				1	2	3	4	4S		HR versus IR/LR	T cells	
[23]	8/8. Primary tumour versus blood	1, 2, 3, 4	FC: % CD3 ⁺ of total single-cell suspension after digestion	2.4 −17.8% (mean 6.7; n = 4) CD3 ⁺	0.4% (n = 1) CD3 ⁺	1.4 −7.1% (mean 4.3; n = 2) CD3 ⁺	0.6%					
[24]	53/53. Primary tumour	1, 2, 3, 4	IHC: % of patients with TIL infiltration	100% (5/ 5)	75% (3/4)	23% (3/ 13)	11% (3/ 28)			2-year OS: 64% in TIL ⁺ versus 11% in TIL [−] . Within stage 3/4: 17% in TIL ⁺ versus 11% in TIL [−] .		
[28]	36/36. Resection tumour	1, 2, 3, 4	IHC CD8: % of patients with CD8 ⁺ infiltration	100% (9/ 9 pts) high CD8 ⁺ in nests and stroma	94% (15/ 16 pts) high CD8 ⁺ in nests and stroma	0% (0/ 6 pts) high CD8 ⁺ in nests and stroma	0% (0/ 4 pts) high CD8 ⁺ in nests and stroma					
[34]	107/107. Primary tumour	1, 2, 3, 4, 4S	RT-qPCR: Vα24-Jα18	72.7% (32/44) Vα24- Jα18	66.7% (16/24) Vα24- Jα18	75% (6/8) Vα24- Jα18	44% (11/ 25) Vα24- Jα18	66.7% (4/6) HR	Lowest levels in HR		iNKT: higher OS in pts with iNKT	
[30]	84/84. Primary tumour	1, 2, 3, 4	IHC CD3, CD4 and CD8				CD3, CD4 and CD8 lowest in stage 4			Higher OS in CD3 ^{hi} and CD4 ^{hi} . CD8: no difference. Higher OS in CD25 ^{hi} and FOXP3 ^{hi} .	No clear association	
[25]	23/23. Primary tumour	N/A	IHC							TIL score correlates with OS months (r _s = 0.69; p < 0.001)		
[32]	77/77. Primary tumour	1, 2, 3, 4, 4S	IHC CD3, CD4 and CD8							Higher OS in CD3 ^{hi} and CD4 ^{hi} . CD8: no difference		
[31]	55/55. Primary tumour and metastasis	HR, NHR	IHC CD8 and PD-1						HR: Higher CD8 ⁺ and more PD-1 on CD8 ⁺			

Table 2 (continued)

Ref	Patients/ samples	Stage	Method	Tumour stage					Risk HR versus IR/LR	Survival analysis		MYCN
				1	2	3	4	4S		T cells	Other lymphocytes ($\gamma\delta$ T, NK, (i)NKT and B)	
[33]	15/15. Primary tumour	LR, IR, HR	IHC CD45 and CD4						HR: Lower CD45 and CD4			Low CD4 infiltration in MYCN-A
[38]	80/80. Primary tumour	1, 2, 3, 4, 4S	IHC CD8									MYCN-A: lower CD8 ⁺ than MYCN- A, also within HR
[44]	41/41. Primary tumour	4	RT-qPCR CD45 and FOXP3							Higher OS and EFS in CD45 ^{hi} and FOXP3 ^{hi} tumours		
[36]	129/129 MYCN- NA. Primary tumour	4	Microarray							5-year EFS not associated with CD3Z, CD4, CD8A, GNLY, IFNG, TBX21, CD40LG, FOXP3, IL13, IL15, IL17	5-year EFS associated with CD1D and NKT	
[43]	98/98. Primary tumour	4	RT-qPCR V α 24-J α 18									MYCN ^{hi} / CCL2 ^{lo} expression predicted absence of iNKT
[33]	TARGET cohort (n = 148). Primary tumour	1, 2, 3, 4	Deconvolution of bulk RNA seq with CIBERSORT							CD4: Higher OS in CD4 ^{hi} , also within MYCN- NA. Th2 (IL-4) but not Th1 (TNF α and IFN γ) » fav prognosis. CD8: no difference		Less CD45 ⁺ , B, CD8 ⁺ , CD4 ⁺ , NK, M \emptyset , mono and DC in MYCN-A than MYCN- NA
[39]	498 primary NBL. Primary tumour	4	Deconvolution of bulk RNA seq with CIBERSORT. Immune signature from [243]							Higher OS in T cell ^{hi} and cytotoxic ^{hi} within stage 4		MYCN-A: less T cells and less cytotoxic signature, also within stage 4 tumours
[38]	TARGET cohort (n = 150). Validation cohort (n = 190). Primary tumour	3, 4, 4S	Deconvolution of bulk RNA seq with CIBERSORT, TCR seq, IHC.							CD4 and CD8 » fav outcome in MYCN-NA with high versus low MYCN expr	NK, $\gamma\delta$ T and B » fav outcome in MYCN-NA with high versus low MYCN expr	MYCN-A: lower immune scores than MYCN-NA, especially cytotoxicity, NK and CD8 ⁺ T, within HR

(continued on next page)

Table 2 (continued)

Ref	Patients/ samples	Stage	Method	Tumour stage					Risk HR versus IR/LR	Survival analysis		MYCN
				1	2	3	4	4S		T cells	Other lymphocytes ($\gamma\delta$ T, NK, (i)NKT and B)	
[37]	TARGET (n = 149). Val (n = 498), (n = 649), (n = 88). Primary tumour	1, 2, 3, 4, 4S	Deconvolution of bulk RNA seq with xCell							CD4 » unfav prognosis and OS. High Th1/ Th2 ratio » OS and EFS. CD8 » fav prognosis and OS	NKT » fav prognosis and OS	Less immune cells in MYCN-A. Higher Th1 and Th2 signatures in MYCN-A.
[244]	Disc (n = 105) and Val (n = 101). Primary tumour	1, 2, 3, 4 (disc) and N/A (val)	WGCNA, GFEA									Immune gene module associated with MYCN- A
[42]	TARGET (n = 160) and Val (n = 498). Primary tumor	1, 2, 3, 4, 4S	Deconvolution of bulk RNA seq with CIBERSORT							Risk score of 9 immune genes (SOCS1, MARCO, KLRK1, IRF7, UNC93B1, IGHV3–20, IGKV1–16, AMH, SECTM1) predicts OS		

enabled more efficient elimination of tumour cells by CTL and NK cells [48]. In line with this reasoning, tumoural MHC-I expression was an important predictor for survival [32]. Thus, different tumour aspects, such as differentiation grade and MHC-I expression, may influence TIL infiltration.

2.2.2. Prognostic value of lymphocyte subsets

While TIL density had a clear predictive value for survival, studies into the prognostic value of TIL subsets yielded conflicting results. On the one hand, two studies reported a beneficial prognostic role for CD8⁺ T cells [31,37], with higher CD8⁺ abundance in HR- compared to LR-neuroblastoma [31], and a higher predicted NKT and CD8⁺ abundance correlating with a favourable prognosis and long-term survival, while CD4⁺ abundance correlated with an unfavourable prognosis [37].

On the other hand, a prevalence of CD4⁺ over CD8⁺ T cells was associated with a better prognosis [30,32,33]. Patients with a high CD4⁺ T cell density had longer OS, DFS, and EFS, while CD8⁺ T cell abundance was not prognostic [30,32,33]. While the former studies emphasise a role for cytotoxic cells in the TME, the latter results suggest that CD4⁺ T cell infiltration may be crucial for the creation of an effective immune response against the tumour.

Reports on CD4⁺ T helper subsets are conflicting as well. One could perhaps expect a primary role for Th1 dominated CD4 responses to create help for CD8⁺

CTL-mediated tumour killing, which is supported by higher Th1/Th2 ratios correlating with better long-term OS and EFS [37], and a higher OS in HR-neuroblastoma patients with a high cytotoxic signature [38,39]. However, others reported that a Th2 oriented CD4⁺ T cell response (defined by IL-4 transcript levels) but not a Th1 oriented response (TNF α and IFN γ levels) was associated with a favourable prognosis [33]. Interestingly, the favourable effect of a Th2 oriented response would be in line with novel insights that B cell infiltration and activation in adult solid cancers yield an important survival benefit [49–51]. The prognostic differences of T cell subsets in neuroblastoma may have resulted from the different techniques, definitions and relatively low sample numbers used. Future studies will have to confirm whether these TIL subsets have a clear prognostic value and role in tumour elimination.

Regulatory T cells (Tregs), often identified by high expression of CD25 and their signature transcription factor FOXP3, play a crucial role in the TME of various tumours and have more often been associated with poor than good survival [52]. This negative effect on survival has been mainly attributed to their immunoregulatory function. In 84 patients with neuroblastoma, a high density of CD25⁺ T cells in the nests and FOXP3⁺ T cells in the septa was associated with better survival [30]. High FOXP3 gene expression in tumour biopsies at diagnosis also predicted a better EFS and OS [44]. Of

note, high expression of CD25 and expression of FOXP3 are not only indicators of Tregs but also of conventional T cell activation (FOXP3 at lower levels than in Tregs). Although Tregs can suppress TIL anti-tumour activity, which would imply a negative effect on survival, it seems that high FOXP3 expression may be a proxy for high T cell infiltration, which is rather, as discussed earlier, beneficial for prognosis.

Not only conventional T cells but also other (immune) cell subsets have been associated with tumour stage. The CD1d-restricted iNKT subset, characterised by expression of V α 24-J α 18, was more abundant in LR- than HR-neuroblastoma and predictive of better OS [34,36]. A combined predictive score, including immune and non-immune cells in the TME, was a potent predictor of OS and EFS, which indicates that not only immune components, but rather the interrelation or interaction between immune and non-immune cells within the TME determines the prognosis of neuroblastoma [37]. Also, others have constructed immune risk scores with predictive value for OS [42].

In conclusion, the prognostic relevance of CD8⁺ and CD4⁺ T cell infiltration, as well as Th polarisation, is still unclear. Future studies will have to point out whether a Th1 driven cytotoxic CTL response or a Th2 driven B cell response, or rather a combination of both, is required for the successful elimination of neuroblastoma tumours.

2.2.3. Relation with MYCN amplification status

Since *MYCN* amplification is an important driver in neuroblastoma and indicative of poor prognosis in low stage tumours [5], several studies have investigated its relation with TIL infiltration and composition. Four computational studies compared the immune composition of *MYCN* amplified (*MYCN-A*) and non-amplified (*MYCN-NA*) tumours by RNA deconvolution in multiple cohorts [33,37–39]. They revealed a strong inverse correlation between *MYCN* amplification and leukocyte infiltration, demonstrating less infiltration of *MYCN-A* tumours by CD8⁺ and CD4⁺ T cells, NK cells, NKT cells, B cells, macrophages, and monocytes (Fig. 1) [33,37–39]. Remarkably, one of the studies reported higher CD4⁺ Th1 and Th2 signatures in *MYCN-A* tumours compared to *MYCN-NA* tumours, which may have resulted from distinct immune definitions used in the different deconvolution methods [37]. While one study did not find an association between TIL infiltration and *MYCN* status [30], the overall computational predictions were confirmed in two IHC-based studies, showing lower CD4⁺ and CD8⁺ T cell infiltration in *MYCN-A* tumours [33,38]. *MYCN* overexpression on RNA level in conjunction with low *CCL2* expression also predicted the absence of iNKT cells [43]. Lastly, *MYCN-A* tumours had significantly lower cytotoxic TIL signatures and NK signatures compared to *MYCN-NA* tumours, indicating that not only the number of cells but also their cytotoxic activity may be lower [38,39].

Since *MYCN* amplification status and tumour stage are closely related, this raises the question whether the correlation with TIL infiltration is driven by *MYCN* amplification or tumour stage. Importantly, even within the group of HR-neuroblastoma, T cell and cytotoxic cell signatures, as well as CD8⁺ infiltration, were lower in *MYCN-A* than *MYCN-NA* tumours [38,39], suggesting that low TIL infiltration is at least partly related to *MYCN* amplification. Overall, these results suggest that *MYCN-A* tumours have a reduced infiltration and activity of TIL compared to *MYCN-NA* tumours, independent of tumour stage.

Not only *MYCN* amplification but also *MYCN* overactivation may affect immune profiles. Within patients with *MYCN-NA* tumours, those with *MYCN* activation signatures had lower immune signatures and worse survival than those without *MYCN* activation. Strikingly, within the subgroup of *MYCN-NA* patients with high *MYCN* activation, those with elevated tumoural activated NK cells, CD8⁺ T cells, and cytolytic signatures showed improved outcome compared to patients with lower immune signatures [38]. These findings suggest that activated infiltrating cytotoxic immune cells can ‘rescue’ patient outcomes within this specific subgroup of *MYCN-NA* HR-neuroblastoma.

Possible explanations for reduced TIL infiltration in *MYCN-A* tumours could be reduced immunogenicity or reduced lymphocyte attraction and activation. Tumour mutational load is an important determinant of tumour immunogenicity, as illustrated by higher TIL infiltration and cytotoxicity in tumours with a higher mutational load [53]. Neuroblastoma tumours typically have a low mutational burden, and therefore, low immunogenicity [15,54,55]. One study reported increased T cell signatures in HR-neuroblastoma with a high compared to low mutation load, which was dependent on *MYCN* status [39]. *MYCN-A* HR-neuroblastoma tumours had significantly less nonsynonymous mutations than *MYCN-NA* tumours [38,39]. However, other studies on neuroblastoma did not find a clear correlation between tumour mutational load and immune signatures, or mutational load and *MYCN* amplification [38,56]. If *MYCN* amplified tumours indeed have lower mutational load, this could partly explain their lower immunogenicity and low TIL infiltration.

Reduced immune cell attraction/activation in *MYCN-A* tumours could also explain reduced TIL infiltration. Layer *et al.* assessed the gene expression of chemotactic and immune-activating mediators in *MYCN-A* and *MYCN-NA* tumours. *MYCN-A* neuroblastoma showed reduced IFN pathway activity and lower expression of cytokine and chemokine genes [39]. However, it is difficult to dissect cause and consequence: while reduced IFN and chemokine expression could hamper T cell infiltration and activation, a reduced presence of TIL in these tissues could also account for lower cytokine and chemokine expression. A causal role

of *MYCN* was suggested by *MYCN* depletion in neuroblastoma cell lines, which enhanced IFN pathway activity, promoted expression of Th1 recruiting chemokines CXCL9 and CXCL10 and increased T cell infiltration [39]. Moreover, downregulation of *MYCN* in a neuroblastoma cell line enhanced expression of activating NK ligands MICA, ULBPs and PVR, resulting in increased NK cell cytolytic activity, while *MYCN* overexpression effectuated the opposite, which corroborated findings in human neuroblastoma tumours [57]. In addition, *MYCN*-A tumours repressed CCL2 expression, an important chemoattractant for lymphocytes [43,58]. Thus, *MYCN* amplification may directly impact the immunogenicity of neuroblastoma tumours and alter their chemokine profile.

Most studies, taken together, suggest that *MYCN*-A neuroblastoma may be less immunogenic to T cell immune surveillance on multiple levels. It would be interesting to investigate in an experimental model whether conditional knockdown of *MYCN* would increase TIL infiltration into tumours *in vivo*, to assess a causal relationship between *MYCN* expression and TIL infiltration. To our knowledge, such a study has not been performed yet.

2.3. Lessons from experimental models

Although fundamental differences in the human and murine immune system should be taken into account when translating results from experimental models to the human situation, several experimental studies with murine models have studied the effector cells involved in neuroblastoma anti-tumour immunity. In a murine A/J model of C1300 neuroblastoma treated intratumourally with activated DC, depletion of CD4⁺ T cells or NK cells caused early outgrowth of tumours, whereas CD8⁺ T cell depletion resulted in later tumour outgrowth similar to undepleted controls [59]. This suggests that CD4⁺ T cells play an important role in DC-mediated anti-tumour immunity, which replicates the association between CD4⁺ T cell infiltration and survival rates in human neuroblastoma.

In syngeneic C57Bl/6 mice injected with a transplantable TH-*MYCN* cell line endogenously expressing GD2 and lacking MHC-I, tumours in immunocompetent mice contained CD4⁺ and CD8⁺ T cells, macrophages and DC. However, the presence of adaptive immunity had limited influence on tumour growth, illustrating the low immunogenicity of neuroblastoma and/or low tumour reactivity of TIL. In contrast, depletion of NK cells resulted in enhanced tumour outgrowth, demonstrating their importance in the anti-neuroblastoma immune response in MHC-I negative tumours [60].

The tumour burden also impacts anti-tumour immunity. In A/J mice bearing subcutaneous NXS2 neuroblastoma, a smaller tumour burden at treatment start

was associated with increased infiltration of NK and CD8⁺ T cells and increased OS [61]. Similarly, in the transgenic TH-*MYCN* mouse model, small ‘early’ tumours had more T cell infiltration and IFN γ expression, while large ‘late’ tumours had more macrophages, DC, and myeloid-derived suppressor cells (MDSC). During cancer progression, the increased infiltration by myeloid cells may contribute to an immunosuppressive environment limiting lymphoid infiltration [62,63]. This suggests that early detection and treatment may be key to exploit the functionality of cytotoxic cells with immunotherapy in an early phase.

3. Challenges and opportunities for immunotherapy

3.1. Tumour reactivity of TIL

While TIL are present in neuroblastoma, the biggest challenge for immunotherapy so far is to generate effective tumour reactivity, which may depend on T cell-intrinsic factors, as well as tumour immunogenicity and immunomodulatory factors in the TME. The latter will be discussed in the next sections. The persistence of neuroblastoma tumours despite T cell infiltration may suggest that these T cells are not effectively recognising and/or killing neuroblastoma tumour cells. Also, the lack of survival differences between patients with HR-neuroblastoma with and without TIL infiltrates in some studies suggests that TIL may be present, but not tumour-specific or not functional [24]. Although the gathered data so far are not conclusive, some indications suggest that TIL can become activated and recognise autologous tumour cells, however, only at low levels (Fig. 2).

3.1.1. Clonality

Reduced clonality of the TCR repertoire is an indication of the antigen-driven expansion of specific T cell clones. A reduced TCR repertoire in TIL compared to PBL (which could be due to disproportional expansion of tumour reactive clones) could therefore hint towards tumour reactivity. Overall, the tumour-infiltrating T cell pool in untreated neuroblastoma was described as polyclonal, with a diverse V α and V β repertoire [21,38,40]. In a small number of patients, increased clonality was observed in TIL compared to PBL, suggesting that in some patients, TIL expansion based on antigen specificity may occur. Since these studies were performed in untreated patients, they do not reflect possible induced tumour reactivity in response to treatment.

3.1.2. Activation

Not only clonality but also the expression of activation markers can indicate tumour reactivity. Although the levels of activation marker CD25 were similar to PBL in one study [64], another study reported higher levels of

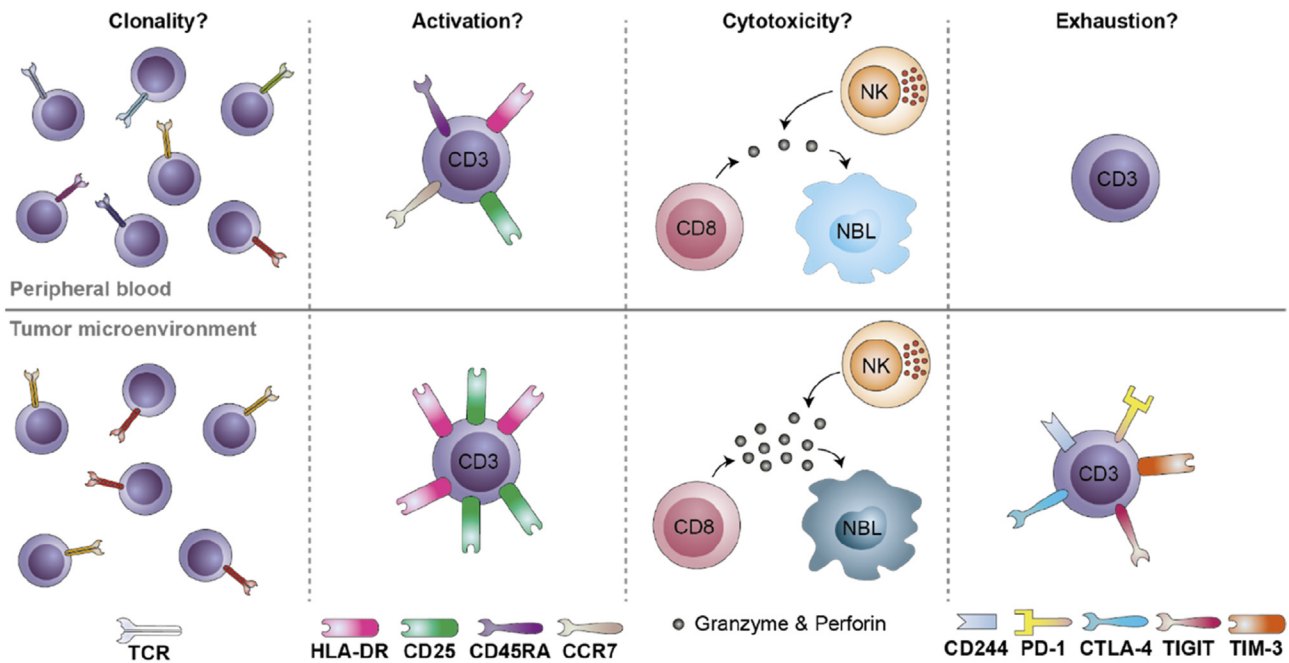


Fig. 2. **Tumour reactivity of TIL in neuroblastoma.** Some indications of tumour reactivity have been described in a small fraction of TIL, including reduced clonality of the T cell receptor repertoire, increased expression of activation markers HLA-DR and CD25, reduced expression of naive T cell markers CD45RA and CCR7, suggesting conversion to a memory state, increased cytotoxicity towards neuroblastoma cells and increased expression of immune checkpoints, which may or may not be associated with an exhausted state.

CD25 in TIL [23]. Others reported that both CD4⁺ and CD8⁺ TIL in neuroblastoma tumours partly expressed CD25 and/or HLA-DR, suggesting an activated state in a fraction of these cells [21]. A small number of T cells in the TME expressed Ki67, indicating active proliferation [23]. In the same detailed IHC-based study, also a redistribution of the CD3 component of the TCR toward the area of contact with the tumour cell was revealed. The authors suggested that the ‘pattern of CD3 distribution, reflecting TCR aggregation [...] and the detection of the proliferation marker Ki67 [...] strongly indicate that tumour recognition takes place *in situ*’ [23]. In line with a more activated state, cells with an effector memory phenotype (CD45RA⁻CCR7⁻) were more frequent in TIL than PBL, and the fraction of naive cells (CD45RA⁺CCR7⁺) was lower in TIL than PBL [23]. Although a predominance of effector memory cells might suggest differentiation upon recognition of a cognate antigen, it is also in line with current insights in the phenotype of human tissue-resident T cells, which is skewed towards the effector memory state in most, even steady-state tissues, precluding the requirement of antigen specificity [65].

Thus, the activation and memory states of TIL may suggest that, at best, a small fraction of T cells actively recognise tumour cells, implying that most are bystander cells. The suggested activation state of TIL could also be reproduced by stimulation of patient PBL with autologous tumour cells in short-term cultures, which increased the proportion of effector memory T cells,

upregulated CD25 expression, stimulated the expression of the Th1 cytokines IFN γ and TNF α , and reduced the expression of TGF β [23]. At least some T cell clones in the peripheral pool, therefore, seem capable of recognising autologous neuroblastoma, which may be indirect evidence that also tumour-infiltrating T cells could contain tumour reactive cells.

3.1.3. Cytotoxicity

As discussed in the previous sections, the presence of a high cytotoxic signature is beneficial for survival in HR-neuroblastoma patients, which underlines the importance of cytotoxic activity over the mere presence of TIL [38,39]. For mounting an effective anti-tumour response, T and NK cells need to be equipped with cytotoxic molecules such as perforin and granzymes to lyse tumour cells. The majority of CD8⁺ T cells in peritumoural stroma expressed perforin [22]. Granzyme B was also detected in TIL, although at low frequency [29]. In patients with HR-neuroblastoma, high gene expression of perforin in biopsies at diagnosis predicted a better EFS and OS, whereas the expression of granzyme B was not prognostic [44]. *In vitro*, TIL showed enhanced cytotoxicity towards neuroblastoma lines compared to PBL [64]. Although this may suggest a degree of neuroblastoma specificity in TIL, it may also be explained by current insights that both tissue- and tumour-resident lymphocytes generally have an enhanced cytotoxic response [65]. Cytotoxicity of TIL against neuroblastoma cell lines was markedly reduced

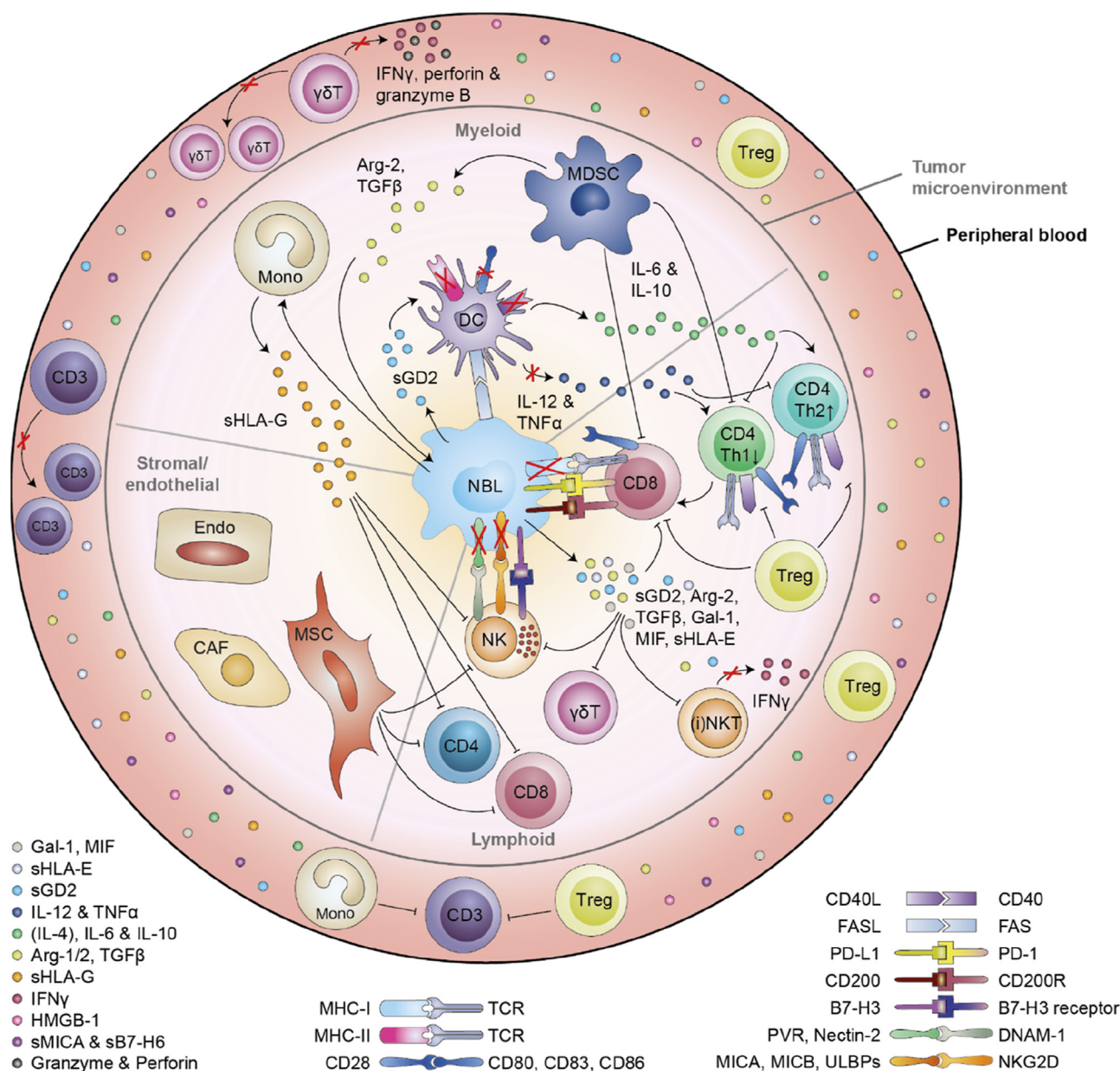


Fig. 3. Immune evasion strategies employed by neuroblastoma. Immune evasion strategies inhibiting T cell responses include down-regulation of MHC-I, and expression of immune checkpoints PD-L1 and CD200. NK cell responses are inhibited by downregulation of ligands for the NK-activating receptors DNAM-1 and NKG2D and increased expression of B7–H3. Neuroblastoma secretes a variety of immunosuppressive molecules, including Gal-1, MIF, sGD2, sHLA-E, TGFβ, sMICA, sB7-H6 and arginase-2, which can impede TIL function. DC function is impaired by sGD2, which leads to decreased expression of MHC-II molecules (antigen presentation), decreased CD28 ligands and CD40 (co-stimulation), reduced Th1-related IL-12 and TNFα production, and increased IL-6 and regulatory IL-10 production. In response to neuroblastoma-derived factors, monocytes produce sHLA-G, which inhibits NK cells and T cells. Also Tregs, MDSC and MSC in the TME can suppress NK cells and T cells. Lastly, immunosuppressive molecules from the TME can reach the circulation and impair the proliferation and cytotoxicity of peripheral T cells and NK cells, while also inducing immunosuppressive monocytes and Tregs, thereby further aggravating lymphocyte dysfunction.

by depletion of CD56⁺ cells from TIL, which recapitulates results in mice [60] and highlights the role of NK cells in anti-neuroblastoma immunity [64]. Since cell lines are derived from allogeneic material, this may not

represent the anti-tumour response in an autologous setting. Coculture of autologous PBL with neuroblastoma reduced tumor growth by ~50% compared to controls, which was similar to the reduction by

allogeneic PBL [66]. *Ex vivo* expanded blood-derived CD8⁺ CTL from patients stimulated with IFN γ -treated neuroblastoma cells, specifically lysed autologous neuroblastoma cells, but not HLA-mismatched neuroblastoma or other autologous cells, suggesting tumour-specificity [67]. *Ex vivo* expanded TIL from neuroblastoma tumours, however, were largely non-reactive to autologous tumour cells, although they did retain the capacity to migrate towards neuroblastoma cell lines [35].

Although TIL may not show sufficient cytotoxic activity *in vivo*, with the right stimulation, they might be trained or unleashed to become reactive to the autologous tumour, which highlights the potential of immunotherapy.

3.1.4. Exhaustion

Another explanation for the ‘ineffective’ presence of TIL in neuroblastoma could be exhaustion, defined as a state of the reduced capacity of cytokine production and usually identified by the combination of typical markers [68]. In neuroblastoma, an upregulation of immune exhaustion genes was found in RNA sequencing data of tumour biopsies. Five out of seven chosen exhaustion markers (CD244, HAVCR2, CTLA4, TIGIT, PDCD1) were significantly higher in tumours with high cytotoxic signatures, possibly indicating that chronic stimulation of T cells with their cognate antigens may have led to an exhausted state [38]. It should be noted, however, that the state of exhaustion and its identification is still subject to discussion in the immunology space [69]. The here mentioned immune checkpoint molecules, which will be further discussed in the next section, do not necessarily indicate exhaustion, but at least point toward the increased activity of immunomodulatory pathways in tumours with a high cytotoxic signature. Moreover, TIL produce a variety of cytokines upon *ex vivo* stimulation with IL-2, which counters the notion of an exhausted state [21]. The relevance of T cell exhaustion in neuroblastoma is, therefore, still debatable.

In conclusion, several studies suggest that, at best, a small fraction of TIL in neuroblastoma may be tumour reactive, although the evidence is not conclusive. The defective anti-tumour response of TIL in neuroblastoma may not only be due to TIL-intrinsic lack of activation but may also result from external immunosuppression by the TME, including immune evasion strategies employed by the tumour.

3.2. Tumour immune evasion strategies

The presence of TIL in neuroblastoma and their possible tumour reactivity support the concept that patients can mount immune responses to neuroblastoma but that these are somehow rendered ineffective. Neuroblastoma tumours employ several strategies to escape

killing by cytotoxic lymphocytes. Among these are the low expression of MHC-I, expression of immune checkpoint molecules, the induction of immunoregulatory myeloid cells and secretion of immunosuppressive mediators (Fig. 3).

3.2.1. Tumour immunogenicity

Neuroblastoma tumours are lowly immunogenic due to their low mutational burden, which is associated with a low generation of neo-antigens [15,54,55]. Tumour-associated antigens, which could be recognised by patrolling T cells, are therefore scarce, although some neuroblastoma-associated immunogenic antigens have been identified [17]. Also, the antigen-presenting machinery, consisting of MHC-I molecules, with which somatic cells constitutively present their intracellular antigens, is not functional in neuroblastoma [16,70,71]. These features render neuroblastoma cells virtually invisible to patrolling CD8⁺ T cells.

Tumoural MHC-I expression in neuroblastoma impacts the immune response. MHC-I expression on tumour cells correlated directly with the density of tumour-infiltrating CD3⁺ T cells, the latter being an important prognostic factor for survival [30]. Apart from CD3 infiltration, high tumoural MHC-I expression was associated with a significantly better OS than low MHC-I expression, and stage 3 and 4 tumours had significantly lower MHC-I expression than stage 1, 2 and 4S tumours [30,32]. The combined presence of MHC-I and/or TIL was even more predictive for survival: all patients with MHC-I^{high}CD3^{high} tumours were alive after >10 years of follow-up, while survival of patients with MHC-I^{low}CD3^{low} tumours was only ~40% [32]. Of note, tumour stage, influencing both MHC-I and CD3 status, likely contributed to this effect since patients were not stratified by tumour stage.

MHC-I expression also depends on *MYCN* amplification status. *MYCN-A* tumours had lower MHC-I expression than *MYCN-NA* tumours, and induction of *MYCN* expression in a *MYCN-NA* neuroblastoma cell line resulted in suppression of MHC-I [38]. Low immunogenicity of *MYCN-A* tumours, as discussed in previous sections, may therefore be a direct result of MHC-I suppression.

The relevance of MHC-I expression was also demonstrated experimentally. Both *in vitro* and *in vivo*, treatment of neuroblastoma models with IFN γ led to increased MHC-I expression and subsequently enhanced killing by CTL [72]. Moreover, a pilot clinical trial among 5 HR-neuroblastoma patients treated with intravenous IFN γ for 5 days resulted in increased MHC-I expression and augmented T cell trafficking in tumours in 2 out of 5 patients. In the other 3 patients, MHC-I was not upregulated, and only very few CD3⁺ T cells were observed [72]. These studies highlight the potential direct *in vitro* and *in vivo* effects of increased MHC-I expression on tumour immunogenicity.

In conclusion, MHC-I and neo-antigen expression are important determinants of tumour immunogenicity and thus T cell infiltration, which affects patient prognosis. MHC-I upregulation should, therefore, be considered as a therapeutic target that may be exploited in combination with additional immunotherapeutic strategies to overcome immune resistance [47,70].

3.2.2. Escaping NK cells

Due to their low MHC-I expression, neuroblastoma cells may not be susceptible to recognition by CTL, but should be rendered vulnerable to NK cells. *In vitro*, NK cells were indeed capable of killing neuroblastoma cells, although pre-activation of isolated NK cells was required in some settings [73,74]. However, in patients, additional escape mechanisms that modulate the balance between activating and inhibitory signals on NK cells seem to protect neuroblastoma from NK-mediated killing (reviewed in Refs. [75]). For example, neuroblastoma tumours have a low expression of ligands (PVR, nectin-2, MICA, MICB and ULBPs) for NK cell-activating receptors DNAM-1 and NKG2D [76,77]. *MYCN* expression can also modulate susceptibility of neuroblastoma to NK-mediated killing: upregulation of *MYCN* in an inducible cell line led to downregulation of NK-activating ligands, resulting in reduced NK-mediated lysis of the tumour cells [57]. However, as demonstrated in multiple studies, NK cell balance can be restored by the anti-GD2 antibody Dinutuximab, which potentially induces NK cell cytotoxicity to neuroblastoma [7,9,12,78,79].

3.2.3. Immune checkpoint molecules

Next to low tumour immunogenicity, the reactivity of TIL towards neuroblastoma may be heavily modulated by the presence of immune checkpoints (IC) in the TME, the importance of which is underlined by the recent breakthroughs achieved by ICI in adult cancers [1].

In pediatric solid cancers, high expression of IC in the TME was associated with poor prognosis [80]. PD-L1 is a potent inhibitor of T cell responses by binding to its receptor PD-1 on effector T cells. Blockade of PD-1/PD-L1 interaction has demonstrated remarkable therapeutic efficacy in various adult cancers by restoring T cell reactivity [81]. Assessment of PD-L1 expression in neuroblastoma has so far yielded conflicting results. IHC-based studies reported variable PD-L1 expression, in 14%, 19%, 35% and >70% of neuroblastoma tumours [27,31,82,83]. High PD-L1 expression was associated with inferior survival and an increased risk of recurrence [27,32,82,83], which was in line with stage 3/4 tumours having higher PD-L1 expression than stage 1, 2 and 4S tumours [32]. Even within HR and stage 4 patients, high PD-L1 expression was associated with inferior survival [27]. While patients with PD-L1^{high}CD3^{low} tumours had the worst prognosis, patients with PD-L1^{low}CD3^{high}

tumours had the best OS [32]. Other studies, however, reported opposite results with lower PD-L1 expression in HR tumours [83,84], or similar PD-L1 expression between HR and non-HR tumours [31]. Reduced PD-L1 expression was also reported in *MYCN*-A tumours [39,83]. Thus, while PD-1 and PD-L1 are expressed in neuroblastoma, their prognostic significance remains uncertain.

Several studies point towards the involvement of the PD-1/PD-L1 axis in TIL infiltration in neuroblastoma. While PD-L1 expression did not correlate with CD45⁺ leukocyte or CD8⁺ T cell infiltration in two studies [31,82], it coincided with and was upregulated by TIL infiltration in another [84]. In neuroblastoma-infiltrated bone marrow (BM), PD-1 was mainly detected on $\alpha\beta$ T cells, $\gamma\delta$ T cells and NK cells, indicating that these may be sensitive to high PD-L1 in the TME [85]. PD-L1 blockade on neuroblastoma cell lines induced CD4⁺ and CD8⁺ T cell activation *in vitro* [86]. In conclusion, the PD-1/PD-L1 checkpoint may contribute to the inhibition of TIL activation in neuroblastoma.

Other IC implicated in neuroblastoma are CD200/CD200R and B7–H3. CD200 was overexpressed on neuroblastoma tumour cells, and its receptor CD200R was (lowly) expressed on CD4⁺ and CD8⁺ TIL [87]. CD200^{high} tumours had lower numbers of CD4⁺ and CD8⁺ TIL, and TIL produced less IFN γ and TNF α [87]. High B7–H3 expression in neuroblastoma correlated with poor EFS [88], and blockade of B7–H3 resulted in enhanced NK cell-mediated killing of neuroblastoma cells *in vitro* [88,89].

The *in vivo* importance of IC activity in neuroblastoma was highlighted in a recently published elegant study demonstrating the formation of human neuroblastoma in mouse-human neural crest chimeras. Although the tumours were extensively infiltrated by tumour reactive mouse CTL, the tumours suppressed immunity by enhancing the expression of PD-L1 and infiltration of Tregs, supporting findings in human neuroblastoma [90]. In conclusion, several IC may be active in neuroblastoma, possibly affecting both T cell and NK cell functions.

3.2.4. Immunosuppressive myeloid and stromal cells

Besides hiding from T cells and NK cells, neuroblastoma employs indirect immunoregulatory mechanisms by chartering myeloid and stromal cells to dampen TIL reactivity.

While T cells have the direct killing capacity, their function depends on antigen presentation and co-stimulation by DC, which are therefore central to anti-tumour immunity. Neuroblastoma can interfere with T cell priming by DC on multiple levels, e.g. by inducing DC apoptosis through the expression of pro-apoptotic factors like FAS ligand [91,92]. FAS ligand expression was associated with high stage tumours independent of *MYCN* status [91], while decreased DC infiltration was

associated with a poor prognosis, underlining their importance for effective anti-tumour immunity [93]. In addition, neuroblastoma can induce DC dysfunction by modulating their antigen-presenting and co-stimulatory capacity through decreasing expression of HLA-DR, co-stimulatory molecules CD40, CD80, CD83, and CD86, and Th1 cytokines IL-12 and TNF α , while increasing IL-6 and regulatory IL-10 [94–96]. These effects were attributed to soluble factors, specifically gangliosides, secreted by the tumour [94–96]. Neuroblastoma-exposed DCs were unable to effectively activate T cells and iNKT cells, which resulted in reduced proliferation, activation and Th1 polarisation, including IFN γ production [94–96], and in some cases could even inhibit T cell proliferation [92]. Thus, neuroblastoma can induce DC dysfunction, which leads to an inability to stimulate effective TIL anti-tumour responses.

Myeloid-derived suppressor cells (MDSCs) are a notorious population of myeloid cells in the TME, which can suppress lymphocyte responses [62,63]. MDSCs accumulated during tumour progression in a murine TH-*MYCN* neuroblastoma model [97], and promoted tumour growth through secretion of reactive oxygen species, arginase-1 and TGF β [98]. Treatment of neuroblastoma-bearing mice with polyphenol E, which reduced the number of MDSC, reinvigorated T cell proliferation and reduced tumour growth [99].

Next to myeloid cells, also stromal cells in the TME may contribute to immunosuppression. Mesenchymal stromal cells (MSC) isolated from neuroblastoma tumours exhibited an extraordinary immunosuppressive capacity on activated T cells, even more than BM-derived MSC [100]. *In vitro* and *in vivo*, NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) against neuroblastoma with anti-GD2 was suppressed by MSC and monocytes [101]. *In vivo* depletion of MSC, monocytes and endothelial cells restored NK cell activity, demonstrating the immunosuppressive capacity of the myeloid and stromal compartments in the TME [101]. In conclusion, neuroblastoma tumours are intermixed with myeloid and stromal cell populations with defective activating functions or enhanced suppressive functions, which can prevent TIL from effectuating an anti-tumour response.

3.2.5. Soluble mediators of immune suppression

Neuroblastoma cells secrete a variety of soluble mediators that can suppress lymphocyte activation, including TGF β , galectin-1, MIF, soluble GD2 (sGD2), and arginase-2.

TGF β , galectin-1 and MIF are well-known immunosuppressive molecules produced by neuroblastoma, which can impair CTL and/or NK cell function [52,102–106]. In experimental neuroblastoma models, NK cell function was directly modulated by TGF β [107], and tumoural galectin-1 or MIF knockdown resulted in

increased T-cell-mediated cytotoxicity, IFN γ secretion, CD4⁺ and CD8⁺ T cell recruitment, and more efficient tumour rejection [104,108,109]. Soluble galectin-1, as well as MIF overexpression, induced T cell apoptosis, inhibited T cell proliferation and inhibited DC maturation [104,110,111]. In patients, high tumoural MIF was associated with lower abundance of CTLs, NKT cells, B cells and DC, and a poor prognosis in stage 4 tumours independent of *MYCN* [112]. Elevated tumoural TGF β was associated with shorter EFS [36]. Thus, TGF β , galectin-1 and MIF secreted by neuroblastoma can reduce TIL anti-tumour activity and affect prognosis.

Surface expression of the ganglioside GD2 is a hallmark of human neuroblastoma. GD2 can be shed in a highly immunosuppressive soluble form, which was detected at >50-fold increased concentrations in patient serum and was particularly suppressive in patients with stage 3/4 tumours [113–115]. sGD2 inhibits T cell proliferation [114,116], and therefore, likely contributes to tumour immune evasion. Anti-GD2 therapy may possibly derive part of its effectivity from blocking sGD2.

Next to immunomodulatory molecules, neuroblastoma produces arginase-2, which reduces levels of arginine – an essential amino acid for lymphocytes – and thereby suppresses T cell proliferation *in vitro* and *in vivo*. High arginase-2 and low arginine were demonstrated in patients with neuroblastoma, both locally and systemically, and arginase activity correlated with poor survival [117]. Mice with a larger tumour burden had lower serum arginine [117], which may explain why those with a large tumour burden showed decreased infiltration of cytotoxic lymphocytes and decreased OS [61]. Thus, arginase-2 activity may have deleterious effects on TIL activity. Also, catecholamines produced by neuroblastoma can have immunoregulatory effects [118,119].

Several other immunosuppressive molecules have been described in neuroblastoma, including sMICA, sB7-H6, sHLA-E, sHLA-G, IL-10 and HMGB1. MICA and B7-H6, neuroblastoma surface-bound ligands for activating receptors on NK cells and CTL, are suppressive when secreted. sMICA was elevated in patient serum, downregulated its receptor NKG2D on CTL and decreased NK-mediated killing of neuroblastoma [77]. Serum sB7-H6 correlated with downregulation of its receptor Nkp30, the occurrence of bone metastases and chemoresistance, and sB7-H6 inhibited NK cell function *in vitro* [120]. Both sMICA and sB7-H6 may thus have relevant immunosuppressive effects in neuroblastoma. HLA-E was highly expressed in neuroblastoma, especially in stage 4 tumours, and inhibited *in vitro* NK cell cytotoxicity while inducing IL-10 and TGF β [121]. sHLA-G was produced by monocytes in the TME in response to neuroblastoma exposure, inhibited NK and CTL function, was increased in neuroblastoma patient

serum and prognostic for relapse [122]. sHLA-G and sHLA-E were highest in BM plasma of patients with metastatic disease [123]. Thus, HLA-E and HLA-G can reduce cytotoxic lymphocyte function and are related to tumour activity. The immunosuppressive HMGB1 was overexpressed in 11% of neuroblastoma patients and was associated with an increased risk of disease progression, relapse and disease-related death [124].

Concluding, a large variety of immunosuppressive factors in the TME can reduce NK cell and CTL function, thereby impairing anti-tumour immunity.

3.2.6. Systemic effects of immunosuppression

The immunosuppressive effects of these soluble factors may not be limited to the TME but may become systemic upon reaching the circulation and induce broad lymphocyte defects. These systemic effects are notable: in untreated neuroblastoma patients, the frequency of circulating T cells is reduced [117], and particularly those with large, invasive tumours and/or metastatic disease, can even present with lymphopenia [125–128]. Not only T cell numbers but also function is decreased. Circulating $\alpha\beta$ T cells and $\gamma\delta$ T cells from untreated neuroblastoma patients had reduced proliferative capacity [117,129], and $\gamma\delta$ T cells showed reduced expression of granzyme B, perforin and IFN γ [129]. Moreover, untreated neuroblastoma patients had increased circulating IL-10, IL-4, IL-6 and reduced IFN γ and IL-12 levels compared to controls, suggesting Th2 dominance with reduced Th1-mediated cytotoxicity [129,130]. Although the lymphocyte defects are likely due to the combination of immunosuppressive signals, the lack of arginine may be crucial, as arginine supplementation reversed the suppressive effect of patient plasma on T cell proliferation [117]. In xenograft studies, T cells showed defective anti-tumour responses as well, both locally and systemically [131].

These defects may be induced directly by the plethora of immunosuppressive signals or indirectly through tumour-educated myeloid cells or Tregs. In untreated neuroblastoma patients, blood-derived myeloid cells suppressed allogeneic T cell responses [117], and circulating Treg frequencies were increased, which normalised after debulking surgery and increased upon tumour progression, suggesting a strong link between tumour activity and Treg induction as an additional immune evasion strategy [130,132]. Defective lymphocyte function was additionally underlined by suboptimal vaccination responses. Importantly, these lymphocyte defects were already present at diagnosis, before treatment, and therefore probably tumour-induced [133]. Subsequent rounds of chemotherapy and radiation therapy likely further reduce T cell numbers and function.

In conclusion, neuroblastoma creates a highly immunosuppressive environment, not only locally in the

tumour, but also systemically, which represents a major challenge for immunotherapeutic interventions.

4. Implications for immunotherapy

4.1. Immunotherapy development

Immunotherapy in neuroblastoma has already proven its efficacy with the introduction of the anti-GD2 antibody Dinutuximab into the standard treatment regimen, which significantly improved survival rates [6,9–13]. Even in patients with relapsed disease, Dinutuximab may have a treatment value [134,135]. The success of Dinutuximab has sparked investigations into combination therapy with cytotoxic compounds (NCT04385277, NCT03794349) [135–137], as well as cellular immunotherapy with (haploidentical) donor NK cells (NCT02573896, NCT03242603, NCT02100891, NCT04211675, NCT01807468) [138,139,148,140–147], the results of which are promising. However, a disadvantage of NK cell-mediated therapy is its inability to induce immunological memory. Patients with minimal residual disease may, therefore, be at risk for relapse upon treatment withdrawal. Induction of a T cell memory response with durable effects may be a desirable, perhaps additional, strategy.

T cells can be engaged, *c. q.* exploited, by cellular therapy with engineered T cells expressing chimeric antigen receptors (CAR) or *ex vivo* activated T cells, by ICI, or vaccination. So far, cellular immunotherapies employing CAR T cells and *ex vivo* expanded cytotoxic lymphocytes have not had overwhelming success in neuroblastoma, with only a fraction of patients achieving measurable responses [149–155]. CAR T cell effectivity is limited by a rapid decline in CAR T cell numbers after infusion, suboptimal potency, a paucity of tumour-specific targets and an immunosuppressive TME [14,156]. Nevertheless, next-generation CAR T cells are currently under investigation in many clinical trials for neuroblastoma, targeted at L1CAM (NCT02311621) [157], B7–H3 (NCT04483778) [158], EGFR (NCT03618381) [159] and GD2 (NCT02919046, NCT02765243, NCT02761915, NCT01822652, NCT03635632, NCT03721068, NCT03294954, NCT02992210, NCT03373097, NCT01953900) [160–169]. Notably, encouraging results of CAR-NKT cell therapy for neuroblastoma were recently reported in an interim analysis [170]. A proof of principle for the exciting possibility of expanding TIL *ex vivo* for reinfusion therapy, which showed remarkable effectivity in melanoma and cervical carcinoma [171,172], was recently provided. Although expanded TIL did not show direct reactivity against neuroblastoma, they produced IFN γ and were able to migrate towards neuroblastoma cell lines [35].

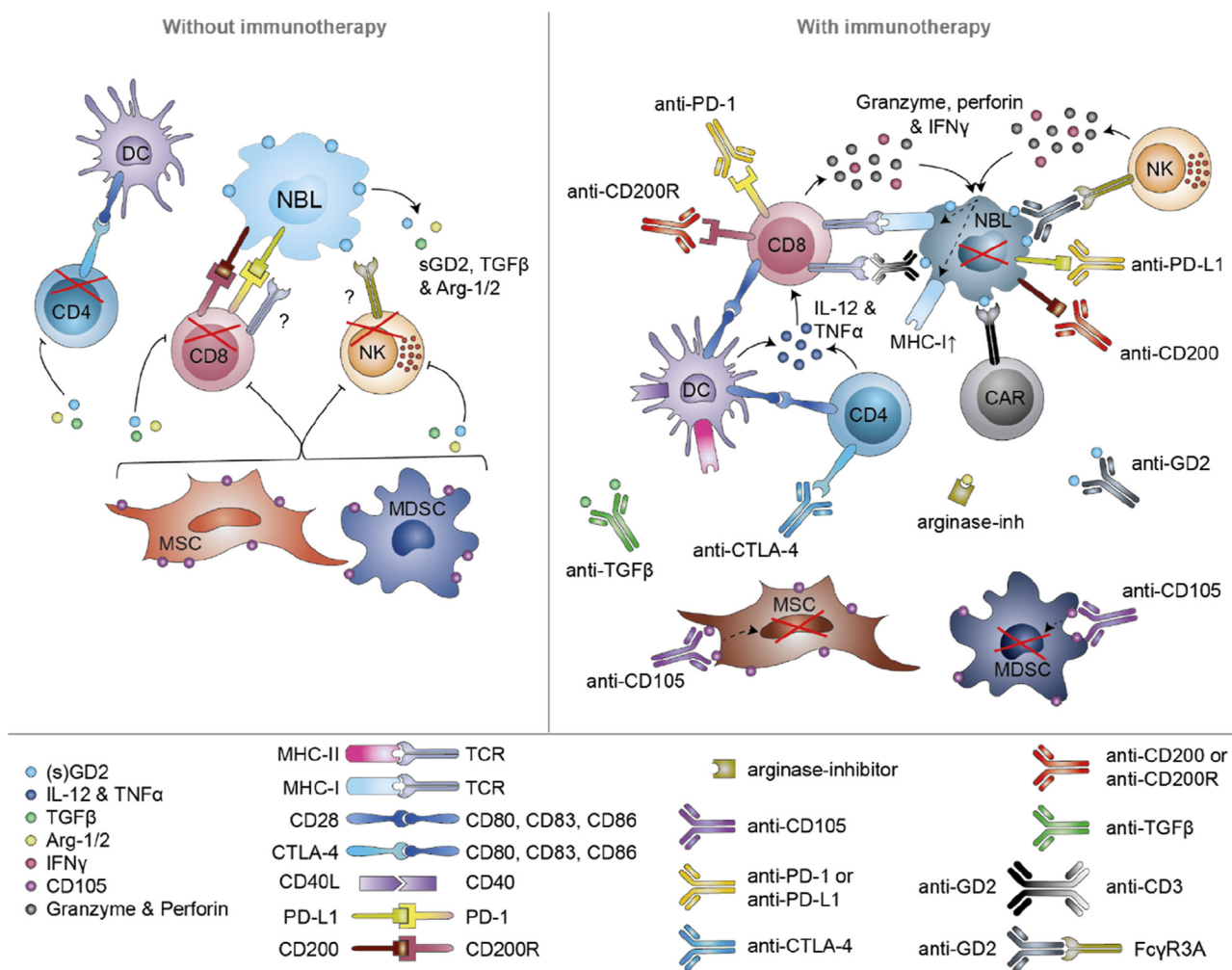


Fig. 4. Immunotherapeutic strategies for neuroblastoma, two or more of which could be combined to increase effectivity. Without immunotherapy, T cells cannot recognise neuroblastoma because of low MHC-I expression, and NK cell activity is hampered by low expression of activating ligands. Soluble immunoregulatory mediators in the TME, MSC and MDSC further inhibit T and NK cell function. Various targets for immunotherapy can be exploited. Checkpoint inhibition can reinvigorate T cell function, bispecific antibodies may induce T cell tumour reactivity, NK cells can be activated with anti-GD2 antibodies and MSC and MDSC can be depleted. The immunosuppressive effect of soluble mediators can be reduced, which will also increase the costimulatory capacity of DC. Successful activation of T and/or NK cells, resulting in secretion of IFN γ , can induce MHC-I expression on neuroblastoma, which further enhances T cell reactivity, thereby creating a positive feedback loop.

As an alternative to cellular immunotherapy, T cell immunity can also be enhanced by ICI, which harnesses naturally occurring anti-tumour T cell responses. Recently, the first clinical trial of ICI among neuroblastoma patients, testing anti-PD-1 antibody Pembrolizumab, reported disappointing results with 4/11 neuroblastoma patients having progressive disease during treatment and none showing a response. Also, in other pediatric solid cancers [80], as well as murine models of neuroblastoma, ICI monotherapy did not show therapeutic effects [84,173–176]. Ongoing studies with ICI in neuroblastoma with Pembrolizumab (NCT02332668) [177], anti-PD1 Nivolumab and/or anti-CTLA4 Ipilimumab (NCT04500548, NCT02304458, NCT03838042, NCT02914405, NCT04412408,

NCT01445379) [178–183], will hopefully provide more insight into their potential efficacy for relapsed neuroblastoma.

For redirecting T cells to the tumour, anti-GD2 x anti-CD3 bispecific antibodies (NCT03860207) [184], as well as *ex vivo* expanded autologous T cells armed with the anti-CD3 x hu3F8 bispecific antibody (GD2Bi, NCT02173093) are currently investigated in phase I/II trials [185].

Lastly, vaccination with tumour antigens or cytokine-producing tumour cells has been explored to induce T cell responses in neuroblastoma, but again with only limited or uncertain long-term responses [29,133,194,186–193]. Although immune activation and even induction of tumour-specific immunity were

observed in most studies, the long-term efficacy remained remarkably low. Novel strategies, including personalised vaccines with self-tumour antigens, may yield increased efficacy of vaccination therapy (NCT04239040, NCT02998983, NCT00911560, NCT04049864) [195–199].

Concluding, the promising results of NK-cell mediated therapies in neuroblastoma are so far not matched by T-cell mediated therapies, which demonstrate only moderate to low effectivity in clinical trials.

4.2. Combination immunotherapy

The limited success of T cell-mediated therapies illustrates the challenges for eliciting T cell reactivity in neuroblastoma, as outlined in the previous paragraphs. It has become clear that not only the natural immune response but also effectivity of immunotherapeutic approaches are hampered by the many immunosuppressive mechanisms at play. Combination immunotherapy, targeting multiple immunomodulatory processes simultaneously, may, therefore, be required (Fig. 4). Especially for cellular therapy or ICI, restoration of an environment conducive to immunity may be necessary to unleash the full T cell potential and increase persistence. This may include restoration of arginine levels [117], depletion of MDSC [101,200], blockade of (soluble) anti-GD2 and TGF β [201], and/or induction of MHC-I expression [202]. Indeed, treatment regimens combining multiple immunotherapeutic strategies, some of which even engage innate and adaptive immunity, were more effective in neuroblastoma patients than monotherapies [139,154,193]. Arginase activity impaired CAR T cell proliferation and cytotoxicity, which could, however, be rescued by inhibition of arginase [117], and resistance to ICI could be overcome by supplementary blockade of TGF β [203,204]. In murine neuroblastoma models, monotherapy with ICI was not effective either, whereas combination therapy with anti-GD2, vaccination, Treg depletion, or myeloid inhibition strongly enhanced anti-tumour immunity [84,173–176,205]. Anti-TGF β improved NK-cell mediated anti-tumour activity [201]. Combining adoptive T cell therapy with ICI to amplify the response is another promising strategy [154,206,207].

Lastly, combining NK cell and T cell-mediated therapies could have the additional benefit of ‘cornering’ the tumour with regard to its MHC-I expression [74]. The combination of anti-PD-1 and anti-GD2 proved encouraging in a recent case report of 2 refractory neuroblastoma patients [208] as well as in a murine neuroblastoma model [205]. However, especially for combination therapy, it will be important to balance effectivity with the risk of immunotoxicity [209].

4.3. Biomarkers predicting immunotherapy response

With only a fraction of patients responding to immunotherapy, the identification of biomarkers predicting therapy responses will be an important aspect of implementing immunotherapy in neuroblastoma. In the adult cancer space, it has become clear that the efficacy of immunotherapy depends on various patient and tumour specific factors, such as tumour mutational load, expression of immune checkpoint molecules, tumour infiltration of T cells and B cells, and presence of lymphoid aggregates [49–51,210–212]. Multifactorial predictive models are being designed to provide more accurate predictions [212–214].

In neuroblastoma, the balance between activating and suppressive NK cells ligands, genotypes of Fc γ -receptors, NKp30, KIR and NKG2D, as well as *MYCN* expression were shown to strongly correlate with the response to anti-GD2 immunotherapy [57,120,215–221]. Biomarkers for T-cell mediated immunotherapy have not been explored yet. In conclusion, personalised (combination) immunotherapy based on response prediction will likely be a valuable approach in neuroblastoma.

5. Conclusion and future perspectives

The success of immunotherapy in various adult cancers has demonstrated its outstanding potency and warrants investigations into the application of existing immunotherapeutics, as well as the development of novel immunotherapeutic interventions for pediatric solid cancers. As discussed in this review, successful implementation of immunotherapy for HR-neuroblastoma is challenging due to the low immunogenicity of HR-neuroblastoma and a large number of immunomodulatory mechanisms at play, both locally and systemically, leading to low numbers and questionable tumour reactivity of TIL. It may, therefore, be an attractive strategy to convert the immunosuppressive environment into an immunostimulating one with targeted therapies. For accelerating novel immunotherapy development for HR-neuroblastoma, some considerations may be taken into account [222].

First, the development of suited *in vitro* and *in vivo* models to study the effect of immune interventions within the context of the TME will be of key importance since cellular crosstalk within the TME heavily shapes the outcome of any intervention. 3D culture systems (e.g. organoids or cell-laden hydrogels) or air-liquid interface cultures that include stromal, endothelial, myeloid, lymphoid and tumoural components, representing the entire TME, could be used to study these interactions [223–225]. Since the murine and human immune systems bear fundamental differences, immunotherapy testing in neuroblastoma models may

require specific, humanised models, preferably with a tumour-autologous immune system [60,226].

Second, to develop targeted interventions, a detailed view of the immunological landscape in neuroblastoma will be essential. So far, published studies show rather conflicting findings, which suggests that the current results may be hampered by low patient numbers and/or suboptimal analysis techniques. Although several studies have confirmed the presence of TIL and their relation to prognosis, the functional specialisation and differentiation of lymphocytes in neuroblastoma are still elusive. For exploiting their cytotoxic functions, e.g. in the context of ICI, it is crucial to gain insights into the phenotype, composition, polarisation, clonality, functionality, and differentiation state in single-cell resolution. Also, the spatial organisation of lymphocytes, e.g. formation of tertiary lymphoid structures, may provide important prognostic and functional clues, as has been demonstrated in adult cancers [22,50,51,227,228]. Moreover, differences in the immune landscape between neuroblastoma patients may serve as biomarkers for the prediction of immunotherapy response.

One of the biggest challenges in neuroblastoma so far is the creation of immunological memory to induce a durable response, preventing relapses. Combination immunotherapy is likely the most promising strategy to overcome low T cell reactivity. Next to combining existing immunotherapies to overcome immune-resistance, novel approaches may improve efficacy. For example, the identification of novel immune checkpoints in the TME with functional relevance, such as TIGIT, TIM-3 and phagocytosis checkpoints [229–231], identification of novel tumour antigens serving as target proteins [232,233], the development of trispecific antibodies for T cell activation [234], reinvigorating myeloid cell function [235,236], personalised CRISPR-engineering of T cells [237,238], CAR-NK cells [239], and nanomedicine [240] are all promising novel approaches currently explored in the adult cancer space.

Also, the timing of immunotherapy may require reconsideration. Early application of ICI in the induction phase may improve efficacy by optimally exploiting the autologous immune response and immunostimulatory effects of chemotherapy [241,242]. This upfront setting will shortly be explored for Dinutuximab in HR-neuroblastoma.

In conclusion, the low immunogenicity of neuroblastoma and widespread immunosuppressive effects of the tumour result in a lack of TIL infiltration, activation and tumour reactivity, which severely hampers the effectivity of current immunotherapeutic strategies. Combination therapy, engaging different cell types or targeting multiple immunological mechanisms simultaneously, may provide more effective therapies. Translation of the here mentioned principles to neuroblastoma, and perhaps pediatric oncology in general, may warrant a deeper understanding of pediatric

tumour immunology and how we should harness it to improve immunotherapy and increase patient survival with optimal quality of life.

Author contributions

Judith Wienke: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization. **Miranda Dierselhuis:** Conceptualization, Investigation, Writing - Original Draft, Writing - Review & Editing. **Lieve Tytgat:** Writing - Review & Editing. **Annette Künkele:** Writing - Review & Editing. **Stefan Nierkens:** Writing - Review & Editing, Supervision. **Jan Molenaar:** Conceptualization, Investigation, Writing - Original Draft, Writing - Review & Editing, Supervision.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] Galluzzi L, Chan TA, Kroemer G, Wolchok JD, Lopez-Soto A. The hallmarks of successful anticancer immunotherapy. *Sci Transl Med* 2018;10. <https://doi.org/10.1126/scitranslmed.aat7807>.
- [2] Casey DL, Cheung N-KV. Immunotherapy of pediatric solid tumors: treatments at a crossroads, with an emphasis on antibodies. *Cancer Immunol Res* 2020;8:161–6. <https://doi.org/10.1158/2326-6066.CIR-19-0692>.
- [3] Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, et al. Neuroblastoma. *Nat Rev Dis Prim* 2016;2:16078. <https://doi.org/10.1038/nrdp.2016.78>.
- [4] Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. *Lancet* 2007;369:2106–20. [https://doi.org/10.1016/S0140-6736\(07\)60983-0](https://doi.org/10.1016/S0140-6736(07)60983-0).
- [5] Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, et al. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N Engl J Med* 1985;313:1111–6. <https://doi.org/10.1056/NEJM198510313131802>.
- [6] Ladenstein R, Potschger U, Valteau-Couanet D, Luksch R, Castel V, Yaniv I, et al. Interleukin 2 with anti-GD2 antibody ch14.18/CHO (dinutuximab beta) in patients with high-risk neuroblastoma (HR-NBL/SIOPEN): a multicentre, randomised, phase 3 trial. *Lancet Oncol* 2018;19:1617–29. [https://doi.org/10.1016/S1470-2045\(18\)30578-3](https://doi.org/10.1016/S1470-2045(18)30578-3).
- [7] Tarek N, Le Ludec J-B, Gallagher MM, Zheng J, Venstrom JM, Chamberlain E, et al. Unlicensed NK cells target neuroblastoma following anti-GD2 antibody treatment. *J Clin Invest* 2012;122:3260–70. <https://doi.org/10.1172/JCI62749>.
- [8] Chen RL, Reynolds CP, Seeger RC. Neutrophils are cytotoxic and growth-inhibiting for neuroblastoma cells with an anti-GD2 antibody but, without cytotoxicity, can be growth-stimulating. *Cancer Immunol Immunother* 2000;48:603–12. <https://doi.org/10.1007/s002620050008>.
- [9] Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, et al. Anti-GD2 antibody with GM-

- CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med* 2010;363:1324–34. <https://doi.org/10.1056/NEJMoa0911123>.
- [10] Simon T, Hero B, Faldum A, Handgretinger R, Schrappe M, Niethammer D, et al. Consolidation treatment with chimeric anti-GD2-antibody ch14.18 in children older than 1 year with metastatic neuroblastoma. *J Clin Oncol* 2004;22:3549–57. <https://doi.org/10.1200/JCO.2004.08.143>.
- [11] Simon T, Hero B, Faldum A, Handgretinger R, Schrappe M, Klingebiel T, et al. Long term outcome of high-risk neuroblastoma patients after immunotherapy with antibody ch14.18 or oral metronomic chemotherapy. *BMC Canc* 2011;11:21. <https://doi.org/10.1186/1471-2407-11-21>.
- [12] Ladenstein R, Potschger U, Valteau-Couanet D, Luksch R, Castel V, Ash S, et al. Investigation of the role of dinutuximab beta-based immunotherapy in the SIOPEX high-risk neuroblastoma 1 trial (HR-NBL1). *Cancers* 2020;12. <https://doi.org/10.3390/cancers12020309>.
- [13] Tas ML, Reedijk AMJ, Karim-Kos HE, Kremer LCM, van de Ven CP, Dierselhuis MP, et al. Neuroblastoma between 1990 and 2014 in The Netherlands: increased incidence and improved survival of high-risk neuroblastoma. *Eur J Canc* 2020;124:47–55. <https://doi.org/10.1016/j.ejca.2019.09.025>.
- [14] Le T-P, Thai T-H. The state of cellular adoptive immunotherapy for neuroblastoma and other pediatric solid tumors. *Front Immunol* 2017;8:1640. <https://doi.org/10.3389/fimmu.2017.01640>.
- [15] Grobner SN, Worst BC, Weischenfeldt J, Buchhalter I, Kleinheinz K, Rudneva VA, et al. The landscape of genomic alterations across childhood cancers. *Nature* 2018;555:321–7. <https://doi.org/10.1038/nature25480>.
- [16] Wolf M, Jungbluth AA, Garrido F, Cabrera T, Meyen-Southard S, Spitz R, et al. Expression of MHC class I, MHC class II, and cancer germline antigens in neuroblastoma. *Cancer Immunol Immunother* 2005;54:400–6. <https://doi.org/10.1007/s00262-004-0603-z>.
- [17] Prigione I, Corrias MV, Airolidi I, Raffaghello L, Morandi F, Bocca P, et al. Immunogenicity of human neuroblastoma. *Ann N Y Acad Sci* 2004;1028:69–80. <https://doi.org/10.1196/annals.1322.008>.
- [18] Raffaghello L, Prigione I, Airolidi I, Camoriano M, Morandi F, Bocca P, et al. Mechanisms of immune evasion of human neuroblastoma. *Canc Lett* 2005;228:155–61. <https://doi.org/10.1016/j.canlet.2004.11.064>.
- [19] Szanto CL, Cornel AM, Vijver SV, Nierkens S. Monitoring immune responses in neuroblastoma patients during therapy. *Cancers* 2020;12. <https://doi.org/10.3390/cancers12020519>.
- [20] Park JA, Cheung N-KV. Targets and antibody formats for immunotherapy of neuroblastoma. *J Clin Oncol Off J Am Soc Clin Oncol* 2020;38:1836–48. <https://doi.org/10.1200/JCO.19.01410>.
- [21] Facchetti P, Prigione I, Ghiotto F, Tasso P, Garaventa A, Pistoia V. Functional and molecular characterization of tumour-infiltrating lymphocytes and clones thereof from a major-histocompatibility-complex-negative human tumour: neuroblastoma. *Cancer Immunol Immunother* 1996;42:170–8. <https://doi.org/10.1007/s002620050267>.
- [22] Coughlin CM, Fleming MD, Carroll RG, Pawel BR, Hogarty MD, Shan X, et al. Immunosurveillance and survivin-specific T-cell immunity in children with high-risk neuroblastoma. *J Clin Oncol* 2006;24:5725–34. <https://doi.org/10.1200/JCO.2005.05.3314>.
- [23] Carlson L-M, De Geer A, Sveinbjornsson B, Orrego A, Martinsson T, Kogner P, et al. The microenvironment of human neuroblastoma supports the activation of tumor-associated T lymphocytes. *OncoImmunology* 2013;2:e23618. <https://doi.org/10.4161/onci.23618>.
- [24] Martin RF, Beckwith JB. Lymphoid infiltrates in neuroblastomas: their occurrence and prognostic significance. *J Pediatr Surg* 1968;3:161–4. [https://doi.org/10.1016/0022-3468\(68\)91005-1](https://doi.org/10.1016/0022-3468(68)91005-1).
- [25] Lauder I, Aherne W. The significance of lymphocytic infiltration in neuroblastoma. *Br J Canc* 1972;26:321–30. <https://doi.org/10.1038/bjc.1972.43>.
- [26] Apps JR, Hasan F, Campus O, Behjati S, Jacques TS, J Sebire N, et al. The immune environment of paediatric solid malignancies: evidence from an immunohistochemical study of clinical cases. *Fetal Pediatr Pathol* 2013;32:298–307. <https://doi.org/10.3109/15513815.2012.754527>.
- [27] Majzner RG, Simon JS, Grosso JF, Martinez D, Pawel BR, Santi M, et al. Assessment of programmed death-ligand 1 expression and tumor-associated immune cells in pediatric cancer tissues. *Cancer* 2017;123:3807–15. <https://doi.org/10.1002/cncr.30724>.
- [28] Rahbar M, Mehrzama M, Karimian M. Tumor infiltrating cytotoxic CD8 T-Cells predict clinical outcome of neuroblastoma in children. *Indian J Med Paediatr Oncol* 2018;39:159–64. https://doi.org/10.4103/ijmpo.ijmpo_78_17.
- [29] Camisaschi C, Renne SL, Beretta V, Rini F, Spagnuolo RD, Tuccitto A, et al. Immune landscape and in vivo immunogenicity of NY-ESO-1 tumor antigen in advanced neuroblastoma patients. *BMC Canc* 2018;18:983. <https://doi.org/10.1186/s12885-018-4910-8>.
- [30] Mina M, Boldrini R, Citti A, Romania P, D'Alicandro V, De Ioris M, et al. Tumor-infiltrating T lymphocytes improve clinical outcome of therapy-resistant neuroblastoma. *OncoImmunology* 2015;4:e1019981. <https://doi.org/10.1080/2162402X.2015.1019981>.
- [31] Chowdhury F, Dunn S, Mitchell S, Mellows T, Ashton-Key M, Gray JC. PD-L1 and CD8+PD1+ lymphocytes exist as targets in the pediatric tumor microenvironment for immunomodulatory therapy. *OncoImmunology* 2015;4:e1029701. <https://doi.org/10.1080/2162402X.2015.1029701>.
- [32] Melaiu O, Mina M, Chierici M, Boldrini R, Jurman G, Romania P, et al. PD-L1 is a therapeutic target of the bromodomain inhibitor JQ1 and, combined with HLA class I, a promising prognostic biomarker in neuroblastoma. *Clin Canc Res* 2017;23:4462–72. <https://doi.org/10.1158/1078-0432.CCR-16-2601>.
- [33] Zhang P, Wu X, Basu M, Dong C, Zheng P, Liu Y, et al. MYCN amplification is associated with repressed cellular immunity in neuroblastoma: an in silico immunological analysis of TARGET database. *Front Immunol* 2017;8:1473. <https://doi.org/10.3389/fimmu.2017.01473>.
- [34] Hishiki T, Mise N, Harada K, Ihara F, Takami M, Saito T, et al. Invariant natural killer T infiltration in neuroblastoma with favorable outcome. *Pediatr Surg Int* 2018;34:195–201. <https://doi.org/10.1007/s00383-017-4189-x>.
- [35] Olle Hurtado M, Wolbert J, Fisher J, Flutter B, Stafford S, Barton J, et al. Tumor infiltrating lymphocytes expanded from pediatric neuroblastoma display heterogeneity of phenotype and function. *PLoS One* 2019;14:e0216373. <https://doi.org/10.1371/journal.pone.0216373>.
- [36] Song L, Asgharzadeh S, Salo J, Engell K, Wu H, Sposto R, et al. Valpha24-invariant NKT cells mediate antitumor activity via killing of tumor-associated macrophages. *J Clin Invest* 2009;119:1524–36. <https://doi.org/10.1172/JCI37869>.
- [37] Zhong X, Zhang Y, Wang L, Zhang H, Liu H, Liu Y. Cellular components in tumor microenvironment of neuroblastoma and the prognostic value. *PeerJ* 2019;7:e8017. <https://doi.org/10.7717/peerj.8017>.
- [38] Wei JS, Kuznetsov IB, Zhang S, Song YK, Asgharzadeh S, Sindiri S, et al. Clinically relevant cytotoxic immune cell signatures and clonal expansion of T-cell receptors in high-risk MYCN -Not-Amplified human neuroblastoma. *Clin Canc Res*

- 2018;24:5673–84. <https://doi.org/10.1158/1078-0432.CCR-18-0599>.
- [39] Layer JP, Kronmüller MT, Quast T, Boorn-Konijnenberg DV, Effern M, Hinze D, et al. Amplification of N-Myc is associated with a T-cell-poor microenvironment in metastatic neuroblastoma restraining interferon pathway activity and chemokine expression. *Oncol Immunology* 2017;6:e1320626. <https://doi.org/10.1080/2162402X.2017.1320626>.
- [40] Valteau D, Scott V, Carcelain G, Hartmann O, Escudier B, Hercend T, et al. T-cell receptor repertoire in neuroblastoma patients. *Canc Res* 1996;56:362–9.
- [41] Slyper M, Porter CBM, Ashenberg O, Waldman J, Drokhyansky E, Wakiro I, et al. A single-cell and single-nucleus RNA-Seq toolbox for fresh and frozen human tumors. *Nat Med* 2020;26:792–802. <https://doi.org/10.1038/s41591-020-0844-1>.
- [42] Jin W, Zhang Y, Liu Z, Che Z, Gao M, Peng H. Exploration of the molecular characteristics of the tumor-immune interaction and the development of an individualized immune prognostic signature for neuroblastoma. *J Cell Physiol* 2020. <https://doi.org/10.1002/jcp.29842>.
- [43] Metelitsa LS, Wu H-W, Wang H, Yang Y, Warsi Z, Asgharzadeh S, et al. Natural killer T cells infiltrate neuroblastomas expressing the chemokine CCL2. *J Exp Med* 2004;199:1213–21. <https://doi.org/10.1084/jem.20031462>.
- [44] Stigliani S, Croce M, Morandi F, Scaruffi P, Rigo V, Carlini B, et al. Expression of FOXP3, CD14, and ARG1 in neuroblastoma tumor tissue from high-risk patients predicts event-free and overall survival. *BioMed Res Int* 2015;2015:347867. <https://doi.org/10.1155/2015/347867>.
- [45] Brodeur GM. Spontaneous regression of neuroblastoma. *Cell Tissue Res* 2018;372:277–86. <https://doi.org/10.1007/s00441-017-2761-2>.
- [46] Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, et al. Advances in risk classification and treatment strategies for neuroblastoma. *J Clin Oncol* 2015;33:3008–17. <https://doi.org/10.1200/JCO.2014.59.4648>.
- [47] Spel L, Nieuwenhuis J, Haarsma R, Stichel E, Bleijerveld OB, Altelaar M, et al. Nedd4-Binding protein 1 and TNFAIP3-interacting protein 1 control MHC-1 display in neuroblastoma. *Canc Res* 2018;78:6621–31. <https://doi.org/10.1158/0008-5472.CAN-18-0545>.
- [48] Carlson L-M, Pahlman S, De Geer A, Kogner P, Levitskaya J. Differentiation induced by physiological and pharmacological stimuli leads to increased antigenicity of human neuroblastoma cells. *Cell Res* 2008;18:398–411. <https://doi.org/10.1038/cr.2008.27>.
- [49] Petitprez F, de Reynies A, Keung EZ, Chen TW-W, Sun C-M, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. *Nature* 2020;577:556–60. <https://doi.org/10.1038/s41586-019-1906-8>.
- [50] Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature* 2020;577:549–55. <https://doi.org/10.1038/s41586-019-1922-8>.
- [51] Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature* 2020;577:561–5. <https://doi.org/10.1038/s41586-019-1914-8>.
- [52] Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Canc* 2012;12:298–306. <https://doi.org/10.1038/nrc3245>.
- [53] McGranahan N, Furness AJS, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 2016;351:1463–9. <https://doi.org/10.1126/science.aaf1490>.
- [54] Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* 2015;348:69–74. <https://doi.org/10.1126/science.aaa4971>.
- [55] Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature* 2017;541:321–30. <https://doi.org/10.1038/nature21349>.
- [56] Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D, et al. The genetic landscape of high-risk neuroblastoma. *Nat Genet* 2013;45:279–84. <https://doi.org/10.1038/ng.2529>.
- [57] Brandetti E, Veneziani I, Melaiu O, Pezzolo A, Castellano A, Boldrini R, et al. MYCN is an immunosuppressive oncogene dampening the expression of ligands for NK-cell-activating receptors in human high-risk neuroblastoma. *Oncol Immunology* 2017;6:e1316439. <https://doi.org/10.1080/2162402X.2017.1316439>.
- [58] Song L, Ara T, Wu H-W, Woo C-W, Reynolds CP, Seeger RC, et al. Oncogene MYCN regulates localization of NKT cells to the site of disease in neuroblastoma. *J Clin Invest* 2007;117:2702–12. <https://doi.org/10.1172/JCI30751>.
- [59] Kawakubo N, Tanaka S, Kinoshita Y, Tajiri T, Yonemitsu Y, Taguchi T. Sequential actions of immune effector cells induced by viral activation of dendritic cells to eliminate murine neuroblastoma. *J Pediatr Surg* 2018;53:1615–20. <https://doi.org/10.1016/j.jpedsurg.2017.08.019>.
- [60] Kroesen M, Nierkens S, Ansems M, Wassink M, Orentas RJ, Boon L, et al. A transplantable TH-MYCN transgenic tumor model in C57Bl/6 mice for preclinical immunological studies in neuroblastoma. *Int J Canc* 2014;134:1335–45. <https://doi.org/10.1002/ijc.28463>.
- [61] Yang RK, Kalogiropoulos NA, Rakhmievich AL, Ranheim EA, Seo S, Kim K, et al. Intratumoral treatment of smaller mouse neuroblastoma tumors with a recombinant protein consisting of IL-2 linked to the hu14.18 antibody increases intratumoral CD8+ T and NK cells and improves survival. *Cancer Immunol Immunother* 2013;62:1303–13. <https://doi.org/10.1007/s00262-013-1430-x>.
- [62] Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol* 2018;19:108–19. <https://doi.org/10.1038/s41590-017-0022-x>.
- [63] Maier B, Leader AM, Chen ST, Tung N, Chang C, LeBerichel J, et al. A conserved dendritic-cell regulatory program limits anti-tumour immunity. *Nature* 2020;580:257–62. <https://doi.org/10.1038/s41586-020-2134-y>.
- [64] Kataoka Y, Matsumura T, Yamamoto S, Sugimoto T, Sawada T. Distinct cytotoxicity against neuroblastoma cells of peripheral blood and tumor-infiltrating lymphocytes from patients with neuroblastoma. *Canc Lett* 1993;73:11–21. [https://doi.org/10.1016/0304-3835\(93\)90182-9](https://doi.org/10.1016/0304-3835(93)90182-9).
- [65] Szabo PA, Miron M, Farber DL. Location, location, location: tissue resident memory T cells in mice and humans. *Sci Immunol* 2019;4. <https://doi.org/10.1126/sciimmunol.aas9673>.
- [66] Hellström IE, Hellström KE, Pierce GE, Bill AH. Demonstration of cell-bound and humoral immunity against neuroblastoma cells. *Proc Natl Acad Sci U S A* 1968;60:1231–8. <https://doi.org/10.1073/pnas.60.4.1231>.
- [67] Sarkar AK, Burlingame SM, Zang YQ, Dulai V, Hicks MJ, Strother DR, et al. Major histocompatibility complex-restricted lysis of neuroblastoma cells by autologous cytotoxic T lymphocytes. *J Immunother* 2001;24:305–11. <https://doi.org/10.1097/00002371-200107000-00006>.
- [68] Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* 2015;15:486–99. <https://doi.org/10.1038/nri3862>.
- [69] Blank CU, Haining WN, Held W, Hogan PG, Kallies A, Lugli E, et al. Defining “T cell exhaustion”. *Nat Rev Immunol* 2019;19:665–74. <https://doi.org/10.1038/s41577-019-0221-9>.

- [70] Spel L, Schiepers A, Boes M. NFkappaB and MHC-1 interplay in neuroblastoma and immunotherapy. *Trends in Cancer* 2018;4: 715–7. <https://doi.org/10.1016/j.trecan.2018.09.006>.
- [71] Raffaghello L, Prigione I, Bocca P, Morandi F, Camoriano M, Gambini C, et al. Multiple defects of the antigen-processing machinery components in human neuroblastoma: immunotherapeutic implications. *Oncogene* 2005;24:4634–44. <https://doi.org/10.1038/sj.onc.1208594>.
- [72] Reid GSD, Shan X, Coughlin CM, Lassoued W, Pawel BR, Wexler LH, et al. Interferon-gamma-dependent infiltration of human T cells into neuroblastoma tumors in vivo. *Clin Canc Res* 2009;15:6602–8. <https://doi.org/10.1158/1078-0432.CCR-09-0829>.
- [73] Main EK, Lampson LA, Hart MK, Kornbluth J, Wilson DB. Human neuroblastoma cell lines are susceptible to lysis by natural killer cells but not by cytotoxic T lymphocytes. *J Immunol* 1985;135:242–6.
- [74] Spel L, Boelens J-J, van der Steen DM, Blokland NJG, van Noesel MM, Molenaar JJ, et al. Natural killer cells facilitate PRAME-specific T-cell reactivity against neuroblastoma. *Oncotarget* 2015;6:35770–81. <https://doi.org/10.18632/oncotarget.5657>.
- [75] Bottino C, Dondero A, Bellora F, Moretta L, Locatelli F, Pistoia V, et al. Natural killer cells and neuroblastoma: tumor recognition, escape mechanisms, and possible novel immunotherapeutic approaches. *Front Immunol* 2014;5:56. <https://doi.org/10.3389/fimmu.2014.00056>.
- [76] Veneziani I, Fruci D, Compagnone M, Pistoia V, Rossi P, Cifaldi L. The BET-bromodomain inhibitor JQ1 renders neuroblastoma cells more resistant to NK cell-mediated recognition and killing by downregulating ligands for NKG2D and DNAM-1 receptors. *Oncotarget* 2019;10:2151–60. <https://doi.org/10.18632/oncotarget.26736>.
- [77] Raffaghello L, Prigione I, Airolidi I, Camoriano M, Levreri I, Gambini C, et al. Downregulation and/or release of NKG2D ligands as immune evasion strategy of human neuroblastoma. *Neoplasia* 2004;6:558–68. <https://doi.org/10.1593/neo.04316>.
- [78] [Internet] ClinicalTrialsgov. High-risk neuroblastoma study 2 of SIOP-Europa-Neuroblastoma (SIOPEN). Bethesda (MD): National Library of Medicine (US); 2019, NCT04221035. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT04221035>. [Accessed 11 August 2020].
- [79] Ozkaynak MF, Gilman AL, London WB, Naranjo A, Diccianni MB, Tenney SC, et al. A comprehensive safety trial of chimeric antibody 14.18 with GM-CSF, IL-2, and isotretinoin in high-risk neuroblastoma patients following myeloablative therapy: children's oncology group study ANBL0931. *Front Immunol* 2018;9:1355. <https://doi.org/10.3389/fimmu.2018.01355>.
- [80] Melaiu O, Lucarini V, Giovannoni R, Fruci D, Gemignani F. News on immune checkpoint inhibitors as immunotherapy strategies in adult and pediatric solid tumors. *Semin Canc Biol* 2020. <https://doi.org/10.1016/j.semcancer.2020.07.001>.
- [81] Shen X, Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. *BMJ* 2018;362: k3529. <https://doi.org/10.1136/bmj.k3529>.
- [82] Zuo S, Sho M, Sawai T, Kanehiro H, Maeda K, Yoshida M, et al. Potential role of the PD-L1 expression and tumor-infiltrating lymphocytes on neuroblastoma. *Pediatr Surg Int* 2020. <https://doi.org/10.1007/s00383-019-04616-9>.
- [83] Saletta F, Vilain RE, Gupta AK, Nagabushan S, Yuksel A, Catchpole D, et al. Programmed death-ligand 1 expression in a large cohort of pediatric patients with solid tumor and association with clinicopathologic features in neuroblastoma. *JCO Precis Oncol* 2017;1–12. <https://doi.org/10.1200/PO.16.00049>.
- [84] Srinivasan P, Wu X, Basu M, Rossi C, Sandler AD. PD-L1 checkpoint inhibition and anti-CTLA-4 whole tumor cell vaccination counter adaptive immune resistance: a mouse neuroblastoma model that mimics human disease. *PLoS Med* 2018;15:e1002497. <https://doi.org/10.1371/journal.pmed.1002497>.
- [85] Dondero A, Pastorino F, Della Chiesa M, Corrias MV, Morandi F, Pistoia V, et al. PD-L1 expression in metastatic neuroblastoma as an additional mechanism for limiting immune surveillance. *OncoImmunology* 2016;5:e1064578. <https://doi.org/10.1080/2162402X.2015.1064578>.
- [86] Boes M, Meyer-Wentrup F. TLR3 triggering regulates PD-L1 (CD274) expression in human neuroblastoma cells. *Canc Lett* 2015;361:49–56. <https://doi.org/10.1016/j.canlet.2015.02.027>.
- [87] Xin C, Zhu J, Gu S, Yin M, Ma J, Pan C, et al. CD200 is overexpressed in neuroblastoma and regulates tumor immune microenvironment. *Cancer Immunol Immunother* 2020. <https://doi.org/10.1007/s00262-020-02589-6>.
- [88] Gregorio A, Corrias MV, Castriconi R, Dondero A, Mosconi M, Gambini C, et al. Small round blue cell tumours: diagnostic and prognostic usefulness of the expression of B7-H3 surface molecule. *Histopathology* 2008;53:73–80. <https://doi.org/10.1111/j.1365-2559.2008.03070.x>.
- [89] Castriconi R, Dondero A, Augugliaro R, Cantoni C, Carnemolla B, Sementa AR, et al. Identification of 4Ig-B7-H3 as a neuroblastoma-associated molecule that exerts a protective role from an NK cell-mediated lysis. *Proc Natl Acad Sci U S A* 2004;101:12640–5. <https://doi.org/10.1073/pnas.0405025101>.
- [90] Cohen MA, Zhang S, Sengupta S, Ma H, Bell GW, Horton B, et al. formation of human neuroblastoma in mouse-human neural crest chimeras. *Cell Stem Cell* 2020;26:579–92. <https://doi.org/10.1016/j.stem.2020.02.001>.
- [91] Gross N, Balmas K, Beretta Brognara C, Tschopp J. Expression of fas (APO-1/CD95) and fas ligand (FasL) in human neuroblastoma. *Med Pediatr Oncol* 2001;36:111–4. [https://doi.org/10.1002/1096-911X\(20010101\)36:1<111::AID-MPO1026>3.0.CO;2-3](https://doi.org/10.1002/1096-911X(20010101)36:1<111::AID-MPO1026>3.0.CO;2-3).
- [92] Chen X, Doffek K, Sugg SL, Shilyansky J. Neuroblastoma cells inhibit the immunostimulatory function of dendritic cells. *J Pediatr Surg* 2003;38:901–5. [https://doi.org/10.1016/s0022-3468\(03\)00119-2](https://doi.org/10.1016/s0022-3468(03)00119-2).
- [93] Redlinger REJ, Mailliard RB, Barksdale EMJ. Neuroblastoma and dendritic cell function. *Semin Pediatr Surg* 2004;13:61–71. <https://doi.org/10.1053/j.sempedsurg.2003.09.009>.
- [94] Walker SR, Redlinger REJ, Barksdale EMJ. Neuroblastoma-induced inhibition of dendritic cell IL-12 production via abrogation of CD40 expression. *J Pediatr Surg* 2005;40:244–50. <https://doi.org/10.1016/j.jpedsurg.2004.09.050>.
- [95] Shurin GV, Shurin MR, Bykovskaia S, Shogan J, Lotze MT, Barksdale EMJ. Neuroblastoma-derived gangliosides inhibit dendritic cell generation and function. *Canc Res* 2001;61:363–9.
- [96] Harada K, Ihara F, Takami M, Kamata T, Mise N, Yoshizawa H, et al. Soluble factors derived from neuroblastoma cell lines suppress dendritic cell differentiation and activation. *Canc Sci* 2019;110:888–902. <https://doi.org/10.1111/cas.13933>.
- [97] Carlson L-M, Kogner P. Neuroblastoma-related inflammation: may small doses of aspirin be suitable for small cancer patients? *OncoImmunology* 2013;2:e24658. <https://doi.org/10.4161/onci.24658>.
- [98] Bianchi G, Vuerich M, Pellegatti P, Marimpietri D, Emionite L, Marigo I, et al. ATP/P2X7 axis modulates myeloid-derived suppressor cell functions in neuroblastoma microenvironment. *Cell Death Dis* 2014;5:e1135. <https://doi.org/10.1038/cddis.2014.109>.
- [99] Santilli G, Piotrowska I, Cantilena S, Chayka O, D'Alicarnasso M, Morgenstern DA, et al. Polyphenon [corrected] E enhances the antitumor immune response in neuroblastoma by inactivating myeloid suppressor cells. *Clin Canc Res* 2013;19:1116–25. <https://doi.org/10.1158/1078-0432.CCR-12-2528>.

- [100] Pelizzo G, Veschi V, Mantelli M, Croce S, Di Benedetto V, D'Angelo P, et al. Microenvironment in neuroblastoma: isolation and characterization of tumor-derived mesenchymal stromal cells. *BMC Canc* 2018;18:1176. <https://doi.org/10.1186/s12885-018-5082-2>.
- [101] Wu H-W, Sheard MA, Malvar J, Fernandez GE, DeClerck YA, Blavier L, et al. Anti-CD105 antibody eliminates tumor microenvironment cells and enhances anti-GD2 antibody immunotherapy of neuroblastoma with activated natural killer cells. *Clin Canc Res* 2019;25:4761–74. <https://doi.org/10.1158/1078-0432.CCR-18-3358>.
- [102] Cohen PS, Letterio JJ, Gaetano C, Chan J, Matsumoto K, Sporn MB, et al. Induction of transforming growth factor beta 1 and its receptors during all-trans-retinoic acid (RA) treatment of RA-responsive human neuroblastoma cell lines. *Canc Res* 1995;55:2380–6.
- [103] Thomas DA, Massagué J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Canc Cell* 2005;8:369–80. <https://doi.org/10.1016/j.ccr.2005.10.012>.
- [104] Soldati R, Berger E, Zenclussen AC, Jorch G, Lode HN, Salatino M, et al. Neuroblastoma triggers an immunoevasive program involving galectin-1-dependent modulation of T cell and dendritic cell compartments. *Int J Canc* 2012;131:1131–41. <https://doi.org/10.1002/ijc.26498>.
- [105] Abe R, Peng T, Sailors J, Bucala R, Metz CN. Regulation of the CTL response by macrophage migration inhibitory factor. *J Immunol* 2001;166:747–53. <https://doi.org/10.4049/jimmunol.166.2.747>.
- [106] Bin Q, Johnson BD, Schauer DW, Casper JT, Orentas RJ. Production of macrophage migration inhibitory factor by human and murine neuroblastoma. *Tumour Biol* 2002;23:123–9. <https://doi.org/10.1159/000064028>.
- [107] Burga RA, Yvon E, Chorvinsky E, Fernandes R, Cruz CRY, Bollard CM. Engineering the TGFbeta receptor to enhance the therapeutic potential of natural killer cells as an immunotherapy for neuroblastoma. *Clin Canc Res* 2019;25:4400–12. <https://doi.org/10.1158/1078-0432.CCR-18-3183>.
- [108] Buchel G, Schulte JH, Harrison L, Batzke K, Schuller U, Hansen W, et al. Immune response modulation by Galectin-1 in a transgenic model of neuroblastoma. *OncoImmunology* 2016;5:e1131378. <https://doi.org/10.1080/2162402X.2015.1131378>.
- [109] Zhou Q, Yan X, Gershan J, Orentas RJ, Johnson BD. Expression of macrophage migration inhibitory factor by neuroblastoma leads to the inhibition of antitumor T cell reactivity in vivo. *J Immunol* 2008;181:1877–86. <https://doi.org/10.4049/jimmunol.181.3.1877>.
- [110] Perillo NL, Pace KE, Seilhamer JJ, Baum LG. Apoptosis of T cells mediated by galectin-1. *Nature* 1995;378:736–9. <https://doi.org/10.1038/378736a0>.
- [111] Yan X, Orentas RJ, Johnson BD. Tumor-derived macrophage migration inhibitory factor (MIF) inhibits T lymphocyte activation. *Cytokine* 2006;33:188–98. <https://doi.org/10.1016/j.cyto.2006.01.006>.
- [112] Cavalli E, Mazzon E, Mammana S, Basile MS, Lombardo SD, Mangano K, et al. Overexpression of macrophage migration inhibitory factor and its homologue D-dopachrome tautomerase as negative prognostic factor in neuroblastoma. *Brain Sci* 2019;9. <https://doi.org/10.3390/brainsci9100284>.
- [113] Li R, Villacreses N, Ladisch S. Human tumor gangliosides inhibit murine immune responses in vivo. *Canc Res* 1995;55:211–4.
- [114] Floutsis G, Ulsh L, Ladisch S. Immunosuppressive activity of human neuroblastoma tumor gangliosides. *Int J Canc* 1989;43:6–9. <https://doi.org/10.1002/ijc.2910430103>.
- [115] Ladisch S, Wu ZL, Feig S, Ulsh L, Schwartz E, Floutsis G, et al. Shedding of GD2 ganglioside by human neuroblastoma. *Int J Canc* 1987;39:73–6. <https://doi.org/10.1002/ijc.2910390113>.
- [116] Li R, Gage D, McKallip R, Ladisch S. Structural characterization and in vivo immunosuppressive activity of neuroblastoma GD2. *Glycoconj J* 1996;13:385–9. <https://doi.org/10.1007/bf00731471>.
- [117] Mussai F, Egan S, Hunter S, Webber H, Fisher J, Wheat R, et al. Neuroblastoma arginase activity creates an immunosuppressive microenvironment that impairs autologous and engineered immunity. *Canc Res* 2015;75:3043–53. <https://doi.org/10.1158/0008-5472.CAN-14-3443>.
- [118] Wood L. Letter: neuroblastoma, immunodeficiency, and catecholamines. *Lancet* 1975;1:1091–2. [https://doi.org/10.1016/s0140-6736\(75\)91866-8](https://doi.org/10.1016/s0140-6736(75)91866-8).
- [119] Elenkov IJ. Effects of catecholamines on the immune response. *NeuroImmune Biology* 2007;7:189–206. [https://doi.org/10.1016/S1567-7443\(07\)00210-4](https://doi.org/10.1016/S1567-7443(07)00210-4). Elsevier.
- [120] Semeraro M, Rusakiewicz S, Minard-Colin V, Delahaye NF, Enot D, Vely F, et al. Clinical impact of the NKp30/B7-H6 axis in high-risk neuroblastoma patients. *Sci Transl Med* 2015;7:283ra55. <https://doi.org/10.1126/scitranslmed.aaa2327>.
- [121] Zhen Z, Guo X, Liao R, Yang K, Ye L, You Z. Involvement of IL-10 and TGF-beta in HLA-E-mediated neuroblastoma migration and invasion. *Oncotarget* 2016;7:44340–9. <https://doi.org/10.18632/oncotarget.10041>.
- [122] Morandi F, Levreri I, Bocca P, Galleni B, Raffaghello L, Ferrone S, et al. Human neuroblastoma cells trigger an immunosuppressive program in monocytes by stimulating soluble HLA-G release. *Canc Res* 2007;67:6433–41. <https://doi.org/10.1158/0008-5472.CAN-06-4588>.
- [123] Morandi F, Pozzi S, Carlini B, Amoroso L, Pistoia V, Corrias MV. Soluble HLA-G and HLA-E levels in bone marrow plasma samples are related to disease stage in neuroblastoma patients. *J Immunol Res* 2016;2016:7465741. <https://doi.org/10.1155/2016/7465741>.
- [124] 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. <https://doi.org/10.1155/2018/7946021>.
- [125] Quinn JJ, Altman AJ. The multiple hematologic manifestations of neuroblastoma. *Am J Pediatr Hematol Oncol* 1979;1:201–5. <https://doi.org/10.1097/00043426-197923000-00002>.
- [126] Scott JP, Morgan E. Coagulopathy of disseminated neuroblastoma. *J Pediatr* 1983;103:219–22. [https://doi.org/10.1016/s0022-3476\(83\)80348-5](https://doi.org/10.1016/s0022-3476(83)80348-5).
- [127] Evans AE, Albo V, D'Angio GJ, Finklestein JZ, Leiken S, Santulli T, et al. Factors influencing survival of children with nonmetastatic neuroblastoma. *Cancer* 1976;38:661–6. [https://doi.org/10.1002/1097-0142\(197608\)38:2<661::aid-cncr2820380206>3.0.co;2-m](https://doi.org/10.1002/1097-0142(197608)38:2<661::aid-cncr2820380206>3.0.co;2-m).
- [128] Chung HS, Higgins GR, Siegel SE, Seeger RC. Abnormalities of the immune system in children with neuroblastoma related to the neoplasm and chemotherapy. *J Pediatr* 1977;90:548–54. [https://doi.org/10.1016/s0022-3476\(77\)80364-8](https://doi.org/10.1016/s0022-3476(77)80364-8).
- [129] Wang X, Mou W, Han W, Xi Y, Chen X, Zhang H, et al. Diminished cytolytic activity of gammadelta T cells with reduced DNAM-1 expression in neuroblastoma patients. *Clin Immunol* 2019;203:63–71. <https://doi.org/10.1016/j.clim.2019.04.006>.
- [130] Morandi F, Croce M, Cangemi G, Barco S, Rigo V, Carlini B, et al. IL-10 and ARG-1 concentrations in bone marrow and peripheral blood of metastatic neuroblastoma patients do not associate with clinical outcome. *J Immunol Res* 2015;2015:718975. <https://doi.org/10.1155/2015/718975>.
- [131] Barr KM, Jing W, Hallett WHD, Gershan JA, Johnson BD.

- Examining T cells at vaccine sites of tumor-bearing hosts provides insights to dysfunctional T-cell immunity. *J Immunother* 2013;36:41–51. <https://doi.org/10.1097/CJI.0b013e318274590e>.
- [132] Tilak T, Sherawat S, Agarwala S, Gupta R, Vishnubhatla S, Bakhshi S. Circulating T-regulatory cells in neuroblastoma: a pilot prospective study. *Pediatr Hematol Oncol* 2014;31:717–22. <https://doi.org/10.3109/08880018.2014.886002>.
- [133] Caruso DA, Orme LM, Amor GM, Neale AM, Radcliff FJ, Downie P, et al. Results of a Phase I study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children with Stage 4 neuroblastoma. *Cancer* 2005;103:1280–91. <https://doi.org/10.1002/cncr.20911>.
- [134] Shusterman S, Naranjo A, Van Ryn C, Hank JA, Parisi MT, Shulkin BL, et al. Antitumor activity and tolerability of hu14.18-IL2 with gmscf and isotretinoin in recurrent or refractory neuroblastoma: a children's oncology group phase II study. *Clin Canc Res* 2019;25:6044–51. <https://doi.org/10.1158/1078-0432.CCR-19-0798>.
- [135] Mody R, Naranjo A, Van Ryn C, Yu AL, London WB, Shulkin BL, et al. Irinotecan-temozolomide with temsirolimus or dinutuximab in children with refractory or relapsed neuroblastoma (COG ANBL1221): an open-label, randomised, phase 2 trial. *Lancet Oncol* 2017;18:946–57. [https://doi.org/10.1016/S1470-2045\(17\)30355-8](https://doi.org/10.1016/S1470-2045(17)30355-8).
- [136] [Internet] ClinicalTrialsgov. Treatment with Dinutuximab in combination with irinotecan and temozolomide after intensive therapy for people with high-risk neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2021, NCT04385277. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT04385277>. [Accessed 11 August 2020].
- [137] [Internet] ClinicalTrialsgov. Irinotecan hydrochloride, temozolomide, and Dinutuximab with or without eflornithine in treating patients with relapsed or refractory neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2019, NCT03794349. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT03794349>. [Accessed 11 August 2020].
- [138] Kanold J, Paillard C, Tchirkov A, Lang P, Kelly A, Halle P, et al. NK cell immunotherapy for high-risk neuroblastoma relapse after haploidentical HSCT. *Pediatr Blood Canc* 2012;59:739–42. <https://doi.org/10.1002/pbc.24030>.
- [139] Federico SM, McCarville MB, Shulkin BL, Sondel PM, Hank JA, Hutson P, et al. A pilot trial of humanized anti-GD2 monoclonal antibody (hu14.18K322A) with chemotherapy and natural killer cells in children with recurrent/refractory neuroblastoma. *Clin Canc Res* 2017;23:6441–9. <https://doi.org/10.1158/1078-0432.CCR-17-0379>.
- [140] Talleur AC, Triplett BM, Federico S, Mamcarz E, Janssen W, Wu J, et al. Consolidation therapy for newly diagnosed pediatric patients with high-risk neuroblastoma using busulfan/melphalan, autologous hematopoietic cell transplantation, anti-GD2 antibody, granulocyte-macrophage colony-stimulating factor, interleukin-2, and hapl. *Biol Blood Marrow Transplant* 2017;23:1910–7. <https://doi.org/10.1016/j.bbmt.2017.07.011>.
- [141] Modak S, Le Luduec J-B, Cheung IY, Goldman DA, Ostrovskaya I, Doubrovina E, et al. Adoptive immunotherapy with haploidentical natural killer cells and Anti-GD2 monoclonal antibody m3F8 for resistant neuroblastoma: results of a phase I study. *OncoImmunology* 2018;7:e1461305. <https://doi.org/10.1080/2162402X.2018.1461305>.
- [142] Choi YB, Son MH, Cho HW, Ma Y, Lee JW, Kang E-S, et al. Safety and immune cell kinetics after donor natural killer cell infusion following haploidentical stem cell transplantation in children with recurrent neuroblastoma. *PLoS One* 2019;14:e0225998. <https://doi.org/10.1371/journal.pone.0225998>.
- [143] Nguyen R, Sahr N, Sykes A, McCarville MB, Federico SM, Sooter A, et al. Longitudinal NK cell kinetics and cytotoxicity in children with neuroblastoma enrolled in a clinical phase II trial. *J Immunother Cancer* 2020;8. <https://doi.org/10.1136/jitc-2019-000176>.
- [144] [Internet] ClinicalTrialsgov. Immunotherapy of relapsed refractory neuroblastoma with expanded NK cells. Bethesda (MD): National Library of Medicine (US); 2018, NCT02573896. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02573896>. [Accessed 11 August 2020].
- [145] [Internet] ClinicalTrialsgov. Immunotherapy of neuroblastoma patients using a combination of anti-GD2 and NK cells. Bethesda (MD): National Library of Medicine (US); 2017, NCT03242603. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT03242603>. [Accessed 11 August 2020].
- [146] [Internet] ClinicalTrialsgov. Phase 2 STIR trial: haploidentical transplant and donor natural killer cells for solid tumors. Bethesda (MD): National Library of Medicine (US); 2014, NCT02100891. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02100891>. [Accessed 11 August 2020].
- [147] [Internet] ClinicalTrialsgov. NK cells infusions with irinotecan, temozolomide, and Dinutuximab. Bethesda (MD): National Library of Medicine (US); 2021, NCT04211675. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT04211675>. [Accessed 11 August 2020].
- [148] [Internet] ClinicalTrialsgov. Haploidentical stem cell transplantation and NK cell therapy in patients with high-risk solid tumors. Bethesda (MD): National Library of Medicine (US); 2013, NCT01807468. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT01807468>. [Accessed 11 August 2020].
- [149] Park JR, Digiusto DL, Slovak M, Wright C, Naranjo A, Wagner J, et al. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. *Mol Ther* 2007;15:825–33. <https://doi.org/10.1038/sj.mt.6300104>.
- [150] Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood* 2011;118:6050–6. <https://doi.org/10.1182/blood-2011-05-354449>.
- [151] Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat Med* 2008;14:1264–70. <https://doi.org/10.1038/nm.1882>.
- [152] Favrot M, Floret D, Michon J, Negrier S, Bouffet E, Coze C, et al. A phase-II study of adoptive immunotherapy with continuous infusion of interleukin-2 in children with advanced neuroblastoma. A report on 11 cases. *Canc Treat Rev* 1989;16(Suppl A):129–42. [https://doi.org/10.1016/0305-7372\(89\)90033-9](https://doi.org/10.1016/0305-7372(89)90033-9).
- [153] Negrier S, Michon J, Floret D, Bouffet E, Gentet JC, Philip I, et al. Interleukin-2 and lymphokine-activated killer cells in 15 children with advanced metastatic neuroblastoma. *J Clin Oncol* 1991;9:1363–70. <https://doi.org/10.1200/JCO.1991.9.8.1363>.
- [154] Heczey A, Louis CU, Savoldo B, Dakhova O, Duret A, Grilley B, et al. CAR T cells administered in combination with lymphodepletion and PD-1 inhibition to patients with neuroblastoma. *Mol Ther* 2017;25:2214–24. <https://doi.org/10.1016/j.ymthe.2017.05.012>.
- [155] Yang L, Ma X, Liu Y-C, Zhao W, Yu L, Qin M, 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;130:3335. https://doi.org/10.1182/blood.V130.Suppl_1.3335.3335.
- [156] Richards RM, Sotillo E, Majzner RG. CAR T cell therapy for neuroblastoma. *Front Immunol* 2018;9:2380. <https://doi.org/10.3389/fimmu.2018.02380>.
- [157] [Internet] ClinicalTrialsgov. Engineered neuroblastoma cellular immunotherapy (ENCIT)-01. Bethesda (MD): National Library of Medicine (US); 2014, NCT02311621. 2000 Feb 29, <https://>

- clinicaltrials.gov/show/NCT02311621. [Accessed 11 August 2020].
- [158] [Internet] ClinicalTrialsgov. B7H3 CAR T Cell Immunotherapy for Recurrent/Refractory Solid Tumors in Children and Young Adults. Bethesda (MD): National Library of Medicine (US); 2020, NCT04483778. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT04483778>. [Accessed 11 August 2020].
- [159] [Internet] ClinicalTrialsgov. EGFR806 CAR T cell immunotherapy for recurrent/refractory solid tumors in children and Young adults. Bethesda (MD): National Library of Medicine (US); 2019, NCT03618381. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT03618381>. [Accessed 11 August 2020].
- [160] [Internet] ClinicalTrialsgov. Study evaluating the efficacy and safety with CAR-T for relapsed or refractory neuroblastoma in children. Bethesda (MD): National Library of Medicine (US); 2016, NCT02919046. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02919046>. [Accessed 11 August 2020].
- [161] [Internet] ClinicalTrialsgov. Anti-GD2 4th generation CART cells targeting refractory and/or recurrent neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2016, NCT02765243. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02765243>. [Accessed 11 August 2020].
- [162] [Internet] ClinicalTrialsgov. A cancer research UK trial of anti-GD2 T-cells (1RG-CART). Bethesda (MD): National Library of Medicine (US); 2013, NCT01822652. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT01822652>. [Accessed 11 August 2020].
- [163] [Internet] ClinicalTrialsgov. 3rd generation GD-2 chimeric antigen receptor and iCaspase suicide safety switch, neuroblastoma, GRAIN. Bethesda (MD): National Library of Medicine (US); 2013, NCT01822652. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT01822652>. [Accessed 11 August 2020].
- [164] [Internet] ClinicalTrialsgov. C7R-GD2.CART cells for patients with relapsed or refractory neuroblastoma and other GD2 positive cancers (GAIL-N). Bethesda (MD): National Library of Medicine (US); 2019, NCT03635632. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT03635632>. [Accessed 11 August 2020].
- [165] [Internet] ClinicalTrialsgov. Study of CAR T-cells targeting the GD2 with IL-15+iCaspase9 for relapsed/refractory neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2019, NCT03721068. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT03721068>. [Accessed 11 August 2020].
- [166] [Internet] ClinicalTrialsgov. GD2 specific CAR and interleukin-15 expressing autologous NKT cells to treat children with neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2018, NCT03294954. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT03294954>. [Accessed 11 August 2020].
- [167] [Internet] ClinicalTrialsgov. Study on GD2 positive solid tumors by 4SCAR-GD2. Bethesda (MD): National Library of Medicine (US); 2016, NCT02992210. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02992210>. [Accessed 11 August 2020].
- [168] [Internet] ClinicalTrialsgov. Anti-GD2 CAR T cells in pediatric patients affected by high risk and/or relapsed/refractory neuroblastoma or other GD2-positive solid tumors. Bethesda (MD): National Library of Medicine (US); 2018, NCT03373097. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT03373097>. [Accessed 11 August 2020].
- [169] [Internet] ClinicalTrialsgov. iC9-GD2-CAR-VZV-CTLs/Refractory or metastatic GD2-positive sarcoma and neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2014, NCT01953900. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT01953900>. [Accessed 11 August 2020].
- [170] Heczey A, Courtney AN, Montalbano A, Robinson S, Liu K, Li M, et al. Anti-GD2 CAR-NKT cells in patients with relapsed or refractory neuroblastoma: an interim analysis. *Nat Med* 2020. <https://doi.org/10.1038/s41591-020-1074-2>.
- [171] Sarnaik A, Khushalani NI, Chesney JA, Lewis KD, Medina TM, Kluger HM, et al. Long-term follow up of lifileucel (LN-144) cryopreserved autologous tumor infiltrating lymphocyte therapy in patients with advanced melanoma progressed on multiple prior therapies. *J Clin Oncol* 2020;38:10006. https://doi.org/10.1200/JCO.2020.38.15_suppl.10006.
- [172] Jazaeri AA, Zsiros E, Amaria RN, Artz AS, Edwards RP, Wenham RM, et al. Safety and efficacy of adoptive cell transfer using autologous tumor infiltrating lymphocytes (LN-145) for treatment of recurrent, metastatic, or persistent cervical carcinoma. *J Clin Oncol* 2019;37:2538. https://doi.org/10.1200/JCO.2019.37.15_suppl.2538.
- [173] Williams EL, Dunn SN, James S, Johnson PW, Cragg MS, Glennie MJ, et al. Immunomodulatory monoclonal antibodies combined with peptide vaccination provide potent immunotherapy in an aggressive murine neuroblastoma model. *Clin Canc Res* 2013;19:3545–55. <https://doi.org/10.1158/1078-0432.CCR-12-3226>.
- [174] Rigo V, Emionite L, Daga A, Astigiano S, Corrias MV, Quintarelli C, et al. Combined immunotherapy with anti-PDL-1/PD-1 and anti-CD4 antibodies cures syngeneic disseminated neuroblastoma. *Sci Rep* 2017;7:14049. <https://doi.org/10.1038/s41598-017-14417-6>.
- [175] Mao Y, Eissler N, Blanc K Le, Johnsen JI, Kogner P, Kiessling R. Targeting suppressive myeloid cells potentiates checkpoint inhibitors to control spontaneous neuroblastoma. *Clin Canc Res* 2016;22:3849–59. <https://doi.org/10.1158/1078-0432.CCR-15-1912>.
- [176] Voeller J, Erbe AK, Slowinski J, Rasmussen K, Carlson PM, Hoefges A, et al. Combined innate and adaptive immunotherapy overcomes resistance of immunologically cold syngeneic murine neuroblastoma to checkpoint inhibition. *J Immunother Cancer* 2019;7:344. <https://doi.org/10.1186/s40425-019-0823-6>.
- [177] [Internet] ClinicalTrialsgov. A study of Pembrolizumab (MK-3475) in pediatric participants with an advanced solid tumor or lymphoma (MK-3475-051/KEYNOTE-051). Bethesda (MD): National Library of Medicine (US); 2015, NCT02332668. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02332668>. [Accessed 11 August 2020].
- [178] [Internet] ClinicalTrialsgov. Testing the combination of two immunotherapy drugs (Nivolumab and Ipilimumab) in children, adolescent, and Young adult patients with relapsed/refractory cancers that have an increased number of genetic changes, the 3CI study. Bethesda (MD): National Library of Medicine (US); 2020, NCT04500548. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT04500548>. [Accessed 11 August 2020].
- [179] [Internet] ClinicalTrialsgov. Nivolumab with or without Ipilimumab in treating younger patients with recurrent or refractory solid tumors or sarcomas. Bethesda (MD): National Library of Medicine (US); 2015, NCT02304458. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02304458>. [Accessed 11 August 2020].
- [180] [Internet] ClinicalTrialsgov. INFORM2 study uses Nivolumab and entinostat in children and adolescents with high-risk refractory malignancies. Bethesda (MD): National Library of Medicine (US); 2019, NCT03838042. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT03838042>. [Accessed 11 August 2020].
- [181] [Internet] ClinicalTrialsgov. Phase I study of investigational medicinal products in children with relapsed/refractory neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2018, NCT02914405. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02914405>. [Accessed 11 August 2020].
- [182] [Internet] ClinicalTrialsgov. Study on sintilimab in stage IV high risk neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2020, NCT04412408. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT04412408>. [Accessed 11 August 2020].

- [183] [Internet] ClinicalTrialsgov. Phase I study of Ipilimumab (Anti-CTLA-4) in children and adolescents with treatment-resistant cancer. Bethesda (MD): National Library of Medicine (US); 2007, NCT01445379. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT01445379>. [Accessed 11 August 2020].
- [184] [Internet] ClinicalTrialsgov. Study of the safety and efficacy of humanized 3F8 bispecific antibody (Hu3F8-BsAb) in patients with relapsed/refractory neuroblastoma, osteosarcoma and other solid tumor cancers. Bethesda (MD): National Library of Medicine (US); 2019, NCT03860207. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT03860207>. [Accessed 11 August 2020].
- [185] [Internet] ClinicalTrialsgov. Activated T cells armed with GD2 bispecific antibody in children and Young adults with neuroblastoma and osteosarcoma. Bethesda (MD): National Library of Medicine (US); 2014, NCT02173093. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02173093>. [Accessed 11 August 2020].
- [186] Krishnadas DK, Shusterman S, Bai F, Diller L, Sullivan JE, Cheerva AC, et al. A phase I trial combining decitabine/dendritic cell vaccine targeting MAGE-A1, MAGE-A3 and NY-ESO-1 for children with relapsed or therapy-refractory neuroblastoma and sarcoma. *Cancer Immunol Immunother* 2015;64:1251–60. <https://doi.org/10.1007/s00262-015-1731-3>.
- [187] Cacciavillano W, Sampor C, Venier C, Gabri MR, de Davila MTG, Galluzzo ML, et al. A phase I study of the anti-idiotypic vaccine racotumomab in neuroblastoma and other pediatric refractory malignancies. *Pediatr Blood Canc* 2015;62:2120–4. <https://doi.org/10.1002/pbc.25631>.
- [188] Kushner BH, Cheung IY, Modak S, Kramer K, Ragupathi G, Cheung N-KV. Phase I trial of a bivalent gangliosides vaccine in combination with beta-glucan for high-risk neuroblastoma in second or later remission. *Clin Canc Res* 2014;20:1375–82. <https://doi.org/10.1158/1078-0432.CCR-13-1012>.
- [189] Russell HV, Strother D, Mei Z, Rill D, Popek E, Biagi E, et al. A phase 1/2 study of autologous neuroblastoma tumor cells genetically modified to secrete IL-2 in patients with high-risk neuroblastoma. *J Immunother* 2008;31:812–9. <https://doi.org/10.1097/CJI.0b013e3181869893>.
- [190] Russell HV, Strother D, Mei Z, Rill D, Popek E, Biagi E, et al. Phase I trial of vaccination with autologous neuroblastoma tumor cells genetically modified to secrete IL-2 and lymphotactin. *J Immunother* 2007;30:227–33. <https://doi.org/10.1097/01.cji.0000211335.14385.57>.
- [191] Rousseau RF, Haight AE, Hirschmann-Jax C, Yvon ES, Rill DR, Mei Z, et al. Local and systemic effects of an allogeneic tumor cell vaccine combining transgenic human lymphotactin with interleukin-2 in patients with advanced or refractory neuroblastoma. *Blood* 2003;101:1718–26. <https://doi.org/10.1182/blood-2002-08-2493>.
- [192] Geiger JD, Hutchinson RJ, Hohenkirk LF, McKenna EA, Yanik GA, Levine JE, et al. Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression. *Canc Res* 2001;61:8513–9.
- [193] Bowman L, Grossmann M, Rill D, Brown M, Zhong WY, Alexander B, et al. IL-2 adenovector-transduced autologous tumor cells induce antitumor immune responses in patients with neuroblastoma. *Blood* 1998;92:1941–9.
- [194] Bowman LC, Grossmann M, Rill D, Brown M, Zhong WY, Alexander B, et al. Interleukin-2 gene-modified allogeneic tumor cells for treatment of relapsed neuroblastoma. *Hum Gene Ther* 1998;9:1303–11. <https://doi.org/10.1089/hum.1998.9.9-1303>.
- [195] Hlavackova E, Pilatova K, Cerna D, Selingerova I, Mudry P, Mazanek P, et al. Dendritic cell-based immunotherapy in advanced sarcoma and neuroblastoma pediatric patients: anti-cancer treatment preceding monocyte harvest impairs the immunostimulatory and antigen-presenting behavior of DCs and manufacturing process outcome. *Front Oncol* 2019;9:1034. <https://doi.org/10.3389/fonc.2019.01034>.
- [196] [Internet] ClinicalTrialsgov. GVAX plus checkpoint blockade in neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2020, NCT04239040. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT04239040>. [Accessed 11 August 2020].
- [197] [Internet] ClinicalTrialsgov. Racotumomab in patients with high-risk neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2016, NCT02998983. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT02998983>. [Accessed 11 August 2020].
- [198] [Internet] ClinicalTrialsgov. Bivalent vaccine with escalating doses of the immunological adjuvant OPT-821. In: Combination with oral β -glucan for high-risk neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2009, NCT00911560. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT00911560>. [Accessed 11 August 2020].
- [199] [Internet] ClinicalTrialsgov. DNA vaccination against neuroblastoma. Bethesda (MD): National Library of Medicine (US); 2019, NCT04049864. 2000 Feb 29, <https://clinicaltrials.gov/show/NCT04049864>. [Accessed 11 August 2020].
- [200] Carlson L-M, Rasmuson A, Idborg H, Segerstrom L, Jakobsson P-J, Sveinbjornsson B, et al. Low-dose aspirin delays an inflammatory tumor progression in vivo in a transgenic mouse model of neuroblastoma. *Carcinogenesis* 2013;34:1081–8. <https://doi.org/10.1093/carcin/bgt009>.
- [201] Tran HC, Wan Z, Sheard MA, Sun J, Jackson JR, Malvar J, et al. TGF β 1 blockade with galunisertib (LY2157299) enhances anti-neuroblastoma activity of the anti-GD2 antibody dinutuximab (ch14.18) with natural killer cells. *Clin Canc Res* 2017;23:804–13. <https://doi.org/10.1158/1078-0432.CCR-16-1743>.
- [202] Cornel AM, Mimpfen IL, Nierkens S. MHC class I down-regulation in cancer: underlying mechanisms and potential targets for cancer immunotherapy. *Cancers* 2020;12. <https://doi.org/10.3390/cancers12071760>.
- [203] Martin CJ, Datta A, Littlefield C, Kalra A, Chapron C, Wawersik S, et al. Selective inhibition of TGF β 1 activation overcomes primary resistance to checkpoint blockade therapy by altering tumor immune landscape. *Sci Transl Med* 2020;12. <https://doi.org/10.1126/scitranslmed.aay8456>.
- [204] Kalbasi A, Ribas A. Tumour-intrinsic resistance to immune checkpoint blockade. *Nat Rev Immunol* 2020;20:25–39. <https://doi.org/10.1038/s41577-019-0218-4>.
- [205] Siebert N, Zumpe M, Juttner M, Troschke-Meurer S, Lode HN. PD-1 blockade augments anti-neuroblastoma immune response induced by anti-GD2 antibody ch14.18/CHO. *OncoImmunology* 2017;6:e1343775. <https://doi.org/10.1080/2162402X.2017.1343775>.
- [206] Mardiana S, Solomon BJ, Darcy PK, Beavis PA. Supercharging adoptive T cell therapy to overcome solid tumor-induced immunosuppression. *Sci Transl Med* 2019;11. <https://doi.org/10.1126/scitranslmed.aaw2293>.
- [207] Grosser R, Cherkassky L, Chintala N, Adusumilli PS. Combination immunotherapy with CAR T cells and checkpoint blockade for the treatment of solid tumors. *Canc Cell* 2019;36:471–82. <https://doi.org/10.1016/j.ccell.2019.09.006>.
- [208] Ehlert K, Hansjuergens I, Zinke A, Otto S, Siebert N, Henze G, et al. Nivolumab and dinutuximab beta in two patients with refractory neuroblastoma. *J Immunother Cancer* 2020;8. <https://doi.org/10.1136/jitc-2020-000540>.
- [209] Armideo E, Callahan C, Madonia L. Immunotherapy for high-

- risk neuroblastoma: management of side effects and complications. *J Adv Pract Oncol* 2017;8:44–55. <https://doi.org/10.6004/jadpro.2017.8.1.4>.
- [210] Auslander N, Zhang G, Lee JS, Frederick DT, Miao B, Moll T, et al. Robust prediction of response to immune checkpoint blockade therapy in metastatic melanoma. *Nat Med* 2018;24:1545–9. <https://doi.org/10.1038/s41591-018-0157-9>.
- [211] Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat Rev Canc* 2019;19:133–50. <https://doi.org/10.1038/s41568-019-0116-x>.
- [212] Liu D, Schilling B, Liu D, Sucker A, Livingstone E, Jerby-Arnon L, et al. Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma. *Nat Med* 2019;25:1916–27. <https://doi.org/10.1038/s41591-019-0654-5>.
- [213] Anagnostou V, Niknafs N, Marrone K, Bruhm DC, White JR, Naidoo J, et al. Multimodal genomic features predict outcome of immune checkpoint blockade in non-small-cell lung cancer. *Nat Can (Que)* 2020;1:99–111. <https://doi.org/10.1038/s43018-019-0008-8>.
- [214] Butner JD, Elganainy D, Wang CX, Wang Z, Chen S-H, Esnaola NF, et al. Mathematical prediction of clinical outcomes in advanced cancer patients treated with checkpoint inhibitor immunotherapy. *Sci Adv* 2020;6:eaay6298. <https://doi.org/10.1126/sciadv.aay6298>.
- [215] Erbe AK, Wang W, Carmichael L, Kim K, Mendonca EA, Song Y, et al. Neuroblastoma patients' KIR and KIR-ligand genotypes influence clinical outcome for dinutuximab-based immunotherapy: a report from the children's oncology group. *Clin Canc Res* 2018;24:189–96. <https://doi.org/10.1158/1078-0432.CCR-17-1767>.
- [216] Forlenza CJ, Boudreau JE, Zheng J, Le Luduec J-B, Chamberlain E, Heller G, et al. KIR3DL1 allelic polymorphism and HLA-B epitopes modulate response to anti-GD2 monoclonal antibody in patients with neuroblastoma. *J Clin Oncol* 2016;34:2443–51. <https://doi.org/10.1200/JCO.2015.64.9558>.
- [217] Siebert N, Jensen C, Troschke-Meurer S, Zumpe M, Juttner M, Ehlert K, et al. Neuroblastoma patients with high-affinity FCGR2A, -3A and stimulatory KIR 2DS2 treated by long-term infusion of anti-GD2 antibody ch14.18/CHO show higher ADCC levels and improved event-free survival. *OncoImmunology* 2016;5:e1235108. <https://doi.org/10.1080/2162402X.2016.1235108>.
- [218] Delgado DC, Hank JA, Kolesar J, Lorentzen D, Gan J, Seo S, et al. Genotypes of NK cell KIR receptors, their ligands, and Fcγ receptors in the response of neuroblastoma patients to Hu14.18-IL2 immunotherapy. *Canc Res* 2010;70:9554–61. <https://doi.org/10.1158/0008-5472.CAN-10-2211>.
- [219] Semeraro M, Rusakiewicz S, Zitvogel L, Kroemer G. Natural killer cell mediated immunosurveillance of pediatric neuroblastoma. *OncoImmunology* 2015;4:e1042202. <https://doi.org/10.1080/2162402X.2015.1042202>.
- [220] Pandey JP, Nambodiri AM. Immunoglobulin GM and FcγRIIIa genotypes influence cytotoxicity of neuroblastoma cells. *J Neuroimmunol* 2014;270:95–7. <https://doi.org/10.1016/j.jneuroim.2014.03.003>.
- [221] Cheung N-KV, Sowers R, Vickers AJ, Cheung IY, Kushner BH, Gorlick R. FCGR2A polymorphism is correlated with clinical outcome after immunotherapy of neuroblastoma with anti-GD2 antibody and granulocyte macrophage colony-stimulating factor. *J Clin Oncol* 2006;24:2885–90. <https://doi.org/10.1200/JCO.2005.04.6011>.
- [222] Hegde PS, Chen DS. Top 10 challenges in cancer immunotherapy. *Immunity* 2020;52:17–35. <https://doi.org/10.1016/j.immuni.2019.12.011>.
- [223] Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, et al. Organoid modeling of the tumor immune microenvironment. *Cell* 2018;175:1972–88. [https://doi.org/10.1016/j-cell.2018.11.021](https://doi.org/10.1016/j.cell.2018.11.021). e16.
- [224] Kock A, Bergqvist F, Steinmetz J, Elfman LHM, Korotkova M, Johnsen JI, et al. Establishment of an in vitro 3D model for neuroblastoma enables preclinical investigation of combined tumor-stroma drug targeting. *Faseb J* 2020. <https://doi.org/10.1096/fj.202000684R>.
- [225] Marrella A, Dondero A, Aiello M, Casu B, Olive D, Regis S, et al. Cell-laden hydrogel as a clinical-relevant 3D model for analyzing neuroblastoma growth, immunophenotype, and susceptibility to therapies. *Front Immunol* 2019;10:1876. <https://doi.org/10.3389/fimmu.2019.01876>.
- [226] Yogev O, Almeida GS, Barker KT, George SL, Kwok C, Campbell J, et al. In vivo modeling of chemoresistant neuroblastoma provides new insights into chemorefractory disease and metastasis. *Canc Res* 2019;79:5382–93. <https://doi.org/10.1158/0008-5472.CAN-18-2759>.
- [227] Jackson HW, Fischer JR, Zanotelli VRT, Ali HR, Mechera R, Soysal SD, et al. The single-cell pathology landscape of breast cancer. *Nature* 2020. <https://doi.org/10.1038/s41586-019-1876-x>.
- [228] Sautes-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat Rev Canc* 2019;19:307–25. <https://doi.org/10.1038/s41568-019-0144-6>.
- [229] Tiragolumab impresses in multiple trials. *Canc Discov* 2020. <https://doi.org/10.1158/2159-8290.CD-NB2020-063>.
- [230] Wolf Y, Anderson AC, Kuchroo VK. TIM3 comes of age as an inhibitory receptor. *Nat Rev Immunol* 2019;20:173–85. <https://doi.org/10.1038/s41577-019-0224-6>.
- [231] Feng M, Jiang W, Kim BYS, Zhang CC, Fu Y-X, Weissman IL. Phagocytosis checkpoints as new targets for cancer immunotherapy. *Nat Rev Canc* 2019;19:568–86. <https://doi.org/10.1038/s41568-019-0183-z>.
- [232] Sussman RT, Rokita JL, Huang K, Raman P, Rathi KS, Martinez D, et al. CAMKV is a candidate immunotherapeutic target in MYCN amplified neuroblastoma. *Front Oncol* 2020;10:302. <https://doi.org/10.3389/fonc.2020.00302>.
- [233] Bosse KR, Raman P, Zhu Z, Lane M, Martinez D, Heitzeneder S, et al. Identification of GPC2 as an oncoprotein and candidate immunotherapeutic target in high-risk neuroblastoma. *Canc Cell* 2017;32:295–309. <https://doi.org/10.1016/j.ccell.2017.08.003>. e12.
- [234] Wu L, Seung E, Xu L, Rao E, Lord DM, Wei RR, et al. Trispecific antibodies enhance the therapeutic efficacy of tumor-directed T cells through T cell receptor co-stimulation. *Nat Can (Que)* 2020;1:86–98. <https://doi.org/10.1038/s43018-019-0004-z>.
- [235] Mayoux M, Roller A, Pulko V, Sammicheli S, Chen S, Sum E, et al. Dendritic cells dictate responses to PD-L1 blockade cancer immunotherapy. *Sci Transl Med* 2020;12. <https://doi.org/10.1126/scitranslmed.aav7431>.
- [236] Sarkar S, Yang R, Mirzaei R, Rawji K, Poon C, Mishra MK, et al. Control of brain tumor growth by reactivating myeloid cells with niacin. *Sci Transl Med* 2020;12. <https://doi.org/10.1126/scitranslmed.aay9924>.
- [237] Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. *Science* 2020;367. <https://doi.org/10.1126/science.aba7365>.
- [238] Roth TL, Li PJ, Blaeschke F, Nies JF, Apathy R, Mowery C, et al. Pooled knockin targeting for genome engineering of cellular immunotherapies. *Cell* 2020. <https://doi.org/10.1016/j.cell.2020.03.039>.
- [239] Wang W, Jiang J, Wu C. CAR-NK for tumor immunotherapy: clinical transformation and future prospects. *Canc Lett* 2020;472:175–80. <https://doi.org/10.1016/j.canlet.2019.11.033>.

- [240] Irvine DJ, Dane EL. Enhancing cancer immunotherapy with nanomedicine. *Nat Rev Immunol* 2020. <https://doi.org/10.1038/s41577-019-0269-6>.
- [241] Topalian SL, Taube JM, Pardoll DM. Neoadjuvant checkpoint blockade for cancer immunotherapy. *Science* 2020;367. <https://doi.org/10.1126/science.aax0182>.
- [242] Galluzzi L, Humeau J, Buqué A, Zitvogel L, Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. *Nat Rev Clin Oncol* 2020. <https://doi.org/10.1038/s41571-020-0413-z>.
- [243] Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* 2013;39:782–95. <https://doi.org/10.1016/j.immuni.2013.10.003>.
- [244] Yang L, Li Y, Wei Z, Chang X. Coexpression network analysis identifies transcriptional modules associated with genomic alterations in neuroblastoma. *Biochim Biophys Acta (BBA) - Mol Basis Dis* 2018;1864:2341–8. <https://doi.org/10.1016/j.bbadis.2017.12.020>.