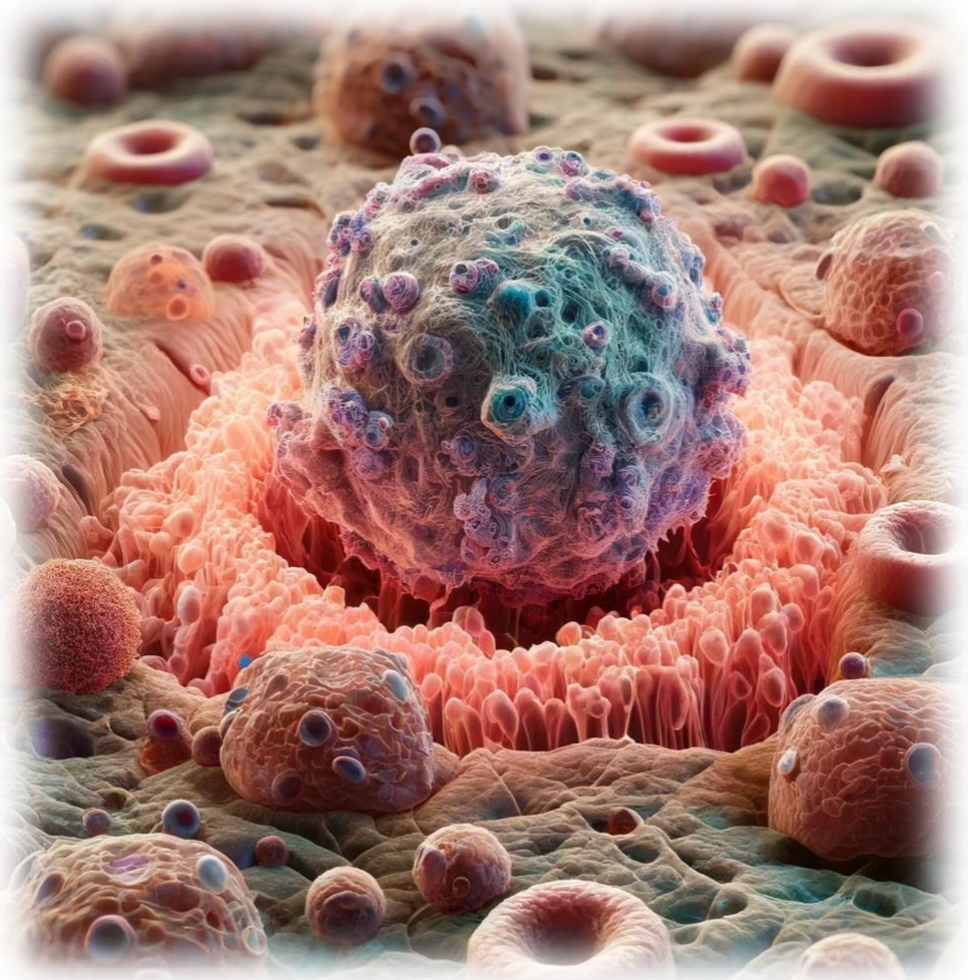
A guide towards immunotherapeutic responsiveness in pediatric tumors:

Assembling the intricate puzzle of the pediatric tumor immune landscape through synthesis of scRNA-sequencing analysis



OpenAI. (2024). Chat GPT4. [Image creation: Tumor, including microenvironment with the focus on immune cells]

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Abstract

In the past decade, immunotherapy has revolutionized the field of cancer treatment. Although successful in certain cancer types, the overall clinical response of immunotherapies is inconsistent and varies significantly among cases. The clinical results of immunotherapy are especially disappointing in pediatric patients, as they fail to respond to standard immunotherapeutic agents relying on a preexisting adaptive antitumor immune response. Additionally, an extreme biological heterogeneity across pediatric cancer types and individual patients creates a unique immune TME for each individual patient, influencing immunotherapeutic treatment response. Distinct tumor immune profiles thus desire specific personalized immunotherapeutic approaches. An accurate characterization of the cellular and molecular components at play in the immune TME of distinct pediatric cancer subtypes is therefore crucial to develop tailored immunotherapeutic applications in pediatric oncology.

In this research, we conducted a meta-analysis on studies analyzing the immune landscape of pediatric cancer types using single-cell RNA sequencing. The aim was to investigate the total immune cell infiltration in solid tumors and in hematologic malignancies. Subsequently, we focused on obtaining distinct immune cell subset fractions as part of the total immune cell population in solid tumors. We systemically searched the PubMed database using the combination of a specific set of search terms. Furthermore, we also briefly searched titles of citations within the acquired publications. This resulted in a total of 12 identified publications that passed eligibility criteria, which were to be used in our meta-analysis. The analysis and synthesis of our obtained data led to a comprehensive overview of the immune TME across pediatric solid cancers, thereby creating a pan-cancer immune atlas. This atlas is eventually ought to guide future immunotherapeutic responsiveness and immunotherapeutic strategy development for pediatric solid tumors. Given the absence of information on functional cell states in our atlas, the potential clinical utility of our pediatric immune atlas has to be taken with caution. Thus, our created pediatric immune atlas as presented in its current form, while valuable, should be interpreted with vigilance if used to directly guide immunotherapeutic decision-making. This meta-analysis should therefore be seen as a foundational step towards a broader understanding of the immunological character of TME's across pediatric solid tumors.

Introduction

In the past decade, immunotherapy has revolutionized the field of cancer treatment. Advances in oncology research have led to the development of several immunotherapies, including adoptive cell therapy, cancer vaccinations and antibody-based immunotherapies[1]. With 9.7 million world-wide cancer deaths per year measured in 2022, the development of these new therapeutic strategies is highly necessary[2]. Although successful in certain cancer subtypes like melanoma, Hodgkin lymphoma and non-small cell lung cancer, the overall clinical response of immunotherapies is inconsistent and varies significantly among cases[1,3]. The cause of this varying clinical response to immunotherapy is the heterogeneity that is present in cancer. Inter- and intra-tumoral heterogeneity underlie therapy resistance and site-specific responses[4]. At the base of this lie a variety of complex and interrelated factors, which are both patient and cancer-type specific. Genetic diversity within tumors causes them to contain multiple cellular subclones with different response profiles. This distinct cellular tumor composition varies across patients, even within the same cancer subtype. Not only within tumors, but also across cancer subtypes molecular signatures differ significantly due to their unique genetic onset. Moreover, the interplay between these genetically distinctive cancer cells with various components of their surrounding tissue creates a unique tumor microenvironment (TME) within individual patients[5]. Immune cells, stromal cells, growth factors and cytokines are all part of the TME and cooperate with malignant cells to promote tumor growth and to create an immunosuppressive environment. In this role, the immune system is thought to be especially important in the development and progression of cancer, since various immune cell subsets each have their individual role in supporting, but also suppressing tumor growth[6]. Since immunotherapy is aimed to target the host's immune system, the uniqueness of each patient's specific immune TME greatly influences immunotherapy response, as distinct tumor immune profiles desire specific personalized immunotherapeutic approaches[3,7].

In pediatric cancers, the immune TME is particularly unique and differs significantly from that seen in adult cancers. This unique TME profile is characterized by a lower mutational burden and different immune cell infiltrates caused by developmental and biological factors intrinsic to children. This also impacts pediatric treatment response. The lower mutational burden results in little neoantigen presence as targets for the immune system to recognize and attack, potentially reducing the efficacy of therapeutic strategies that rely on antigen presentation[7–10]. Even though the immune landscape of pediatric tumors is increasingly being investigated, it has been relatively unexplored compared to adult cancers. This is also reflected in the relatively poor clinical success of immunotherapies in common pediatric cancers, as opposed to the impressive clinical results that several immunotherapeutic strategies have shown in adult cancers[11,12]. For instance, PD-1 and PD-L1 immune checkpoint inhibitors, which have resulted in remained remission in multiple adult cancers, did not have significant clinical effect in most common pediatric cancers[13–15]. Furthermore, adoptive cell transfer attempts did not meet desired clinical expectations either in many cases[12,16,17]. Accordingly, the current arsenal of immunotherapeutic strategies available in pediatric cancers is not as broad as that in adult cancers. This indicates that challenges lie ahead. It however does not imply that immunotherapy does not hold great promise for pediatric oncology. With several FDA-approved immunotherapeutic strategies available for childhood cancers, and multiple immunotherapeutic strategies under evaluation in clinical trials, this shows that the use of immunotherapy in pediatric oncology is still in the early stages of development and has yet to reach its potential[18]. The limited approved immunotherapeutic agents currently include targeted antibodies (blinatumomab, dinutuximab, rituximab, ipilimumab and pembrolizumab) and one CAR-T cell therapy (tisagenlecleucel), with applications being mostly limited to blood cancers like leukemia and lymphoma, and in some cases solid tumors, like neuroblastoma[18].

It is evident that there is a lack of effective immunotherapeutic agents to treat pediatric cancers, and in particular solid tumors. The development of solid tumor targeting therapies encounters challenges like restricted tumor accessibility due to physical barriers, a more complex and immunosuppressive

TME and a lower neoantigen load[19]. This underscores the necessity of the development of immunotherapies that are specifically tailored to the unique immune TME of pediatric patients to ensure effective treatment. An accurate characterization of the cellular and molecular components at play in the immune TME of distinct pediatric cancer subtypes is therefore crucial to develop tailored immunotherapeutic applications. Furthermore, a thorough understanding of the immune TME is also essential to help identify new prognostic and predictive biomarkers to ultimately guide patient specific immunotherapeutic responsiveness.

In this research, we conducted a meta-analysis on studies analyzing the immune landscape of distinct pediatric cancer types using single-cell RNA sequencing (scRNA-seq). We aimed at studies focusing on scRNA-seq as this is technique is critical to characterize the TME, allowing researchers to gain a detailed understanding of the specific types of immune cells present in malignancies, including their states and molecular interactions at the single cell level. The unique immune signatures associated with different types of pediatric cancers can be envisioned this way[20]. This meta-analysis leads to a comprehensive overview of the immune TME across pediatric cancers, thereby creating a pan-cancer immune atlas. This created atlas will enable the guiding of future treatment responsiveness and novel immunotherapy development in pediatric oncology. Since available immunotherapy for pediatric solid tumors is especially lacking, this review ultimately converges on the immune landscape of pediatric solid tumors in specific.

Materials and Methods

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[21].

Search Strategy

For this analysis we tried to identify all available publications which analyzed the immune profile of pediatric cancer types using scRNA-seq. For pediatric solid tumors this translates to scRNA-seq analysis of the immune TME of tumor tissue samples, and for pediatric blood tumors this translates to scRNA-seq of blood or bone marrow samples. The purpose was to obtain the fractions as part of the total number of cells in the tumor samples, and the fractions as part of the total number of immune cells in the tumor samples, for the following distinct immune cell types: T cells (in general), regulatory T cells (Treg), CD4+ T cells, CD8+ T cells, gamma delta T cells (gd T cells), natural killer cells (NK cells), B cells, myeloid cells (in general) macrophages, M1 macrophages, M2 macrophages, microglia cells, dendritic cells (DC) and monocytes. To fulfill this purpose, we systemically searched the PubMed database in April 2024 using the combination of search terms as provided in supplementary data 1. Furthermore, we also briefly searched titles of citations within the acquired publications. All obtained publications were first screened on title, abstract and on cohort age. Moreover, duplicates were removed as well. Hereafter, the remaining articles were thoroughly assessed for eligibility. This literature research was conducted by one author.

Eligibility criteria

To be eligible, several inclusion criteria were determined. This entailed that the publication was only included if the authors, (1) analyzed the immune profile of distinct pediatric cancer samples (for solid tumors the immune TME of tumor samples, for blood tumors the immune profile of blood samples or bone marrow samples), (2) immune cell analysis comprised one or more of the following specific cell types; T cells (in general), regulatory T cells (Treg), CD4+ T cells, CD8+ T cells, gamma delta T cells (gd T cells), natural killer cells (NK cells), B cells, myeloid cells (in general) macrophages, M1 macrophages, M2 macrophages, microglia cells, dendritic cells (DC) and monocytes, (3) analysis was performed based

on data acquired by scRNA-seq, (4) patient age at time of sampling was <26 years old. Publications were excluded if they did not meet these criteria, or if no exact data on immune cell numbers or fractions were present or legible from graphs in the publication, either in the article itself or after data request by mail (with no response before the 28th of May 2024). Besides, publications that used raw scRNA-seq data from another primary article (within our obtained publications) and did not produce new findings were excluded as well.

Bias assessment

For assessing the risk of bias in our retrieved publications several factors must be considered. Heterogeneity in the used experimental methods within these publications may affect scRNA-seq results and therefore need to be evaluated. Firstly, the sampling protocol can introduce bias, as different methods of sample taking can lead to variability in size of samples (para-tumor tissue included or not) and spatial orientation of the sample (tumor center or periphery) affecting the immune cell representation of the analyzed biopsy. Furthermore, treatment regimen of the included patients in each study needs to be assessed because prior treatment can modulate the immune TME in diverse ways[22]. Technological variability among publications also forms a risk for bias. The use of different platforms like 10x Genomics or SMART-Seq has impact on the detection sensitivity and detection rate of genes, influencing sequencing depth. Additionally, inconsistency in scRNA-seq data analysis forms a big risk for bias as well. Mainly the choice of cluster markers and their expression levels are of great importance, as variability in these factors among publications can lead to inaccurate and heterogenous cell type identification[20]. Finally, cohort size is a critical factor to assess among our retrieved publications, as this directly affects the statistical power, reliability and generalizability of the study.

Data analysis and synthesis method

From the obtained publications, we aimed to extract the available fraction data or exact numbers of distinct immune cell subsets investigated, together with the total number of immune cells and the total number of cells in the samples that were analyzed. For the presentation of our results, we first compared the fraction of total immune cells in total cells present within tumor samples among all retrieved cancer subtypes. Furthermore, we investigate individual immune cell subset fractions of total immune cells in the TME among solid tumor cancer sub types to create a pediatric pan-cancer immune atlas for solid tumors. The results of publications covering the same cancer subtypes will first be synthesized before including these distinct subtypes for pan cancer comparison of the immune TME. For this synthesis we will normalize results by taking cohorts size into account. Each specific immune cell fraction present in the obtained publications will be weighed on patient cohort size, being directly proportional to the number of the cohort. All other previously mentioned risk factors that could introduce bias will not be quantified in the synthesis of the results within cancer subtypes. Reason for this is the expected limited obtainable exact data and method information published for identical cancer subtypes, due to the relatively new field of pediatric TME analysis by scRNA-seq. Quantitative integration by weighing heavy on the absence or variability in information regarding previously mentioned risk factors in our obtained publication, therefore may result in a disproportionate effect on the synthesized results when integrating small numbers of publications. Also, directly quantifying the effect of unreported or distinct variables in tumor sampling or scRNA-technology use is extremely challenging.

Results

Study selection

As shown in the preferred reporting items for systematic reviews and meta-analysis (PRISMA) flow diagram (figure 1), our search strategy in the PubMed database resulted in 95 identified studies. After screening on title, abstract and on whether cohorts included pediatric patients, 51 studies were excluded due to undesired content. Of the 44 potentially relevant studies, 10 studies were included based on the eligibility criteria. Simultaneously, we also identified 3 potentially relevant studies from citations within our PubMed retrieved publications. Only 2 of these were eligible for further inclusion. In the end, this resulted in a total of 12 identified publications which passed eligibility criteria that were to be used in our meta-analysis[23–34].

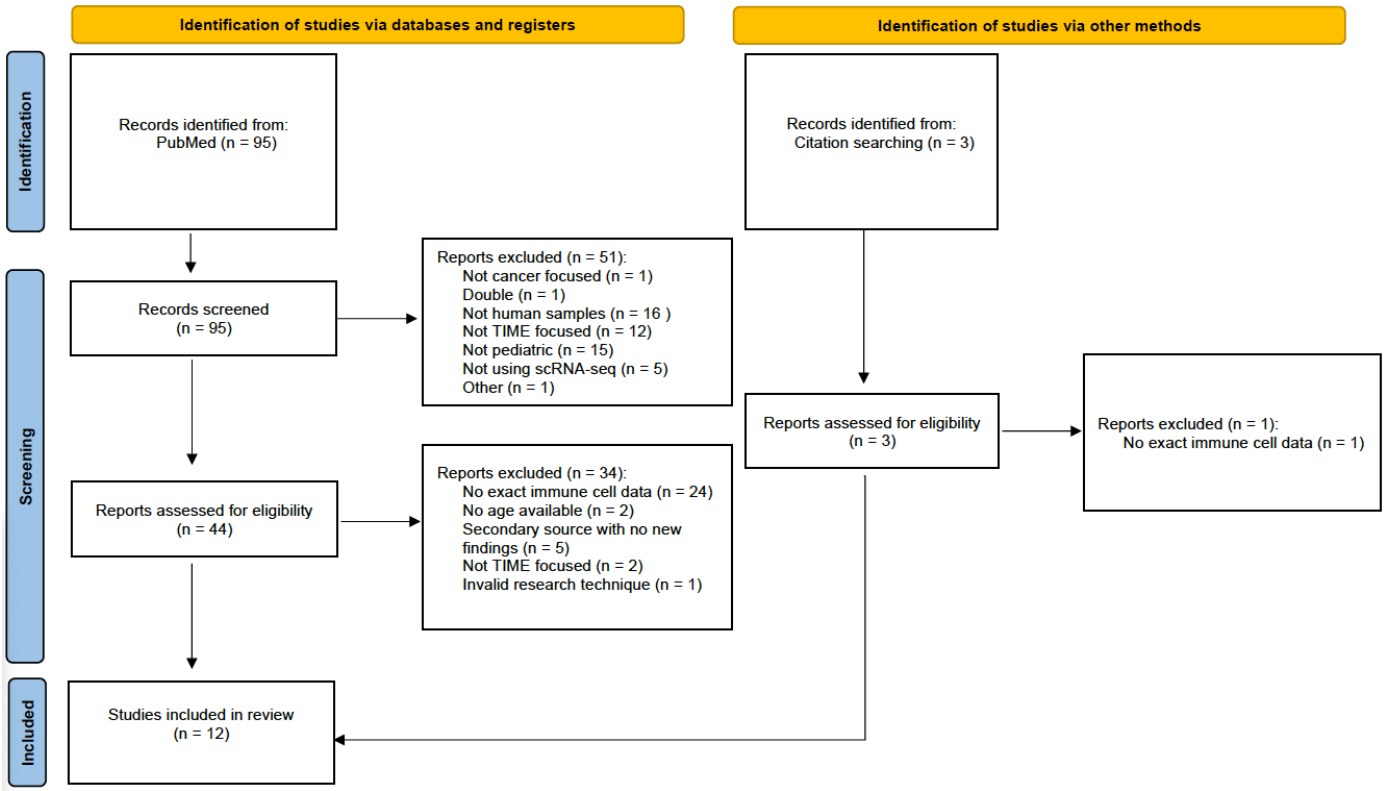

Figure 1: PRISMA flow diagram summarizing the search and selection process for publications included in this study.

Characterization of included publications

The 12 obtained studies, conducted between 2021 and 2024, investigated several distinct pediatric cancers, comprising 7 solid tumor subtypes, and 4 leukemia subtypes. Regarding solid tumors, we obtained 3 studies on glioma's, which we can divide in high-grade glioma's, encompassing several subtypes like glioblastoma and diffuse midline glioma, and low-grade gliomas. Another publication investigating a solid brain tumor comprised medulloblastoma. Furthermore, 2 studies included the analysis of neuroblastoma, and the other studies on solid tumors investigated one tumor type each, namely hepatoblastoma, rhabdomyosarcoma and Ewing sarcoma. For leukemia's, we obtained one study which included two leukemia subtypes, B/myeloid mixed phenotype acute leukemia (B/My MPAL) and T/myeloid mixed phenotype acute leukemia (T/My MPAL), one study which encompassed B-ALL, and one study which focused on ALL with KMT2A-r aberrations in specific. Treatment protocols before sampling and cohort size were notably heterogenous across all obtained studies. Important details and characteristics of the included studies can be found in table 1.

	TUMOR TYPE	SUBTYPE	COHORT SIZE < 26Y	SAMPLE SITE	TREATMENT
DESISTO ET AL. 2024	Glioma	High-grade (3/4)	19	Brain	N/A
ZAHEDI ET AL. 2024	Glioma	Low-Grade	23	Brain	Mixed, 1 patient dabrafenib and 22 patients non-treatment
ABDELFATTAH ET AL. 2022	Glioma	Glioblastoma	1	Brain	N/A
SONG ET AL. 2022	Hepatoblastoma	-	9	Liver	Chemotherapy
MUMME ET AL. 2023	Leukemia	B/My MPAL	4	Bone marrow	Non-treatment
	Leukemia	T/My MPAL	5	Bone marrow	Mixed, 1 patient treatment (N/A) and 4 patients non-treatment
CHEN ET AL. 2022	Leukemia	ALL (KMT2A-r)	18	Peripheral blood	AALL15P1 trial
LIN ET AL. 2022	Leukemia	B-ALL	7	Bone marrow	Mixed, non-treatment & Chemotherapy (7 paired sample pairs)
RIEMONDY ET AL. 2021	Medulloblastoma	-	28	Brain	Non-treatment
WIENKE ET AL. 2024	Neuroblastoma	INNS 3/4	19	Multiple tumor sites	Mixed, non-treatment and chemotherapy
VERHOEVEN ET AL. 2022	Neuroblastoma	-	16	Multiple tumor sites	Mixed, non-treatment and chemotherapy
DEMARTINO ET AL. 2023	Sarcoma	Rhabdomyosarcoma	17	Multiple tumor sites	Mixed, non-treatment and treatment (N/A)
VISSER ET AL. 2023	Sarcoma	Ewing Sarcoma	11	Bone	Mixed, non-treatment and treatment (N/A)

Table 1: Main details and characteristics of all included studies.

Total immune cell fraction in pediatric cancers.

To investigate the overall immune cell infiltration in solid tumors and the total immune cell fraction in bone marrow or peripheral blood samples in hematologic malignancies, we divided the total immune cell number of all samples by the total number of cells in all samples for each pediatric cancer type. To obtain the high-grade glioma overall immune cell infiltration, we first merged the data of both independent studies on high-grade gliomas. This also applies to the two studies on Neuroblastoma. The immune cell fraction of the total amount of cells in the analyzed samples are shown in figure 2 for each independent pediatric cancer subtype.

The analysis of these samples across our obtained studies reveals significant variability in total immune cell fractions within tumor samples across distinct pediatric tumor types, ranging from 0,92 in B-ALL to 0,11 in T/My MPAL[27,29]. Even when distinguishing between leukemia subtypes and solid tumors, heterogeneity in immune cell fractions was clearly visible within both groups. Regarding leukemia subtypes, B-ALL demonstrated the highest immune cell fraction (0,92), which was tremendously higher than the immune cell fractions of T/My MPAL (0,11), B/My MPAL (0,12) and ALL (KMT2A-r aberration) (0,28). Solid tumors also demonstrated a wide range of immune cell infiltration, with fractions peaking at 0,76 in low-grade gliomas and immune infiltration being at the lowest in medulloblastoma, with a total immune cell fraction of 0,12. Remarkable is that both these extremes belong to solid brain tumors. This disparity across pediatric tumor types, but also within similarly classified tumor types, highlights

the profound variability in immune cell dynamics in the TME of pediatric cancers. It is however of importance to bring the nuance to these results that treatment regimen of patients included in our obtained studies differ among these studies. This importance lies in the fact that certain treatments influence and interact with the immune TME, possibly leading to biased fraction outcomes[35].

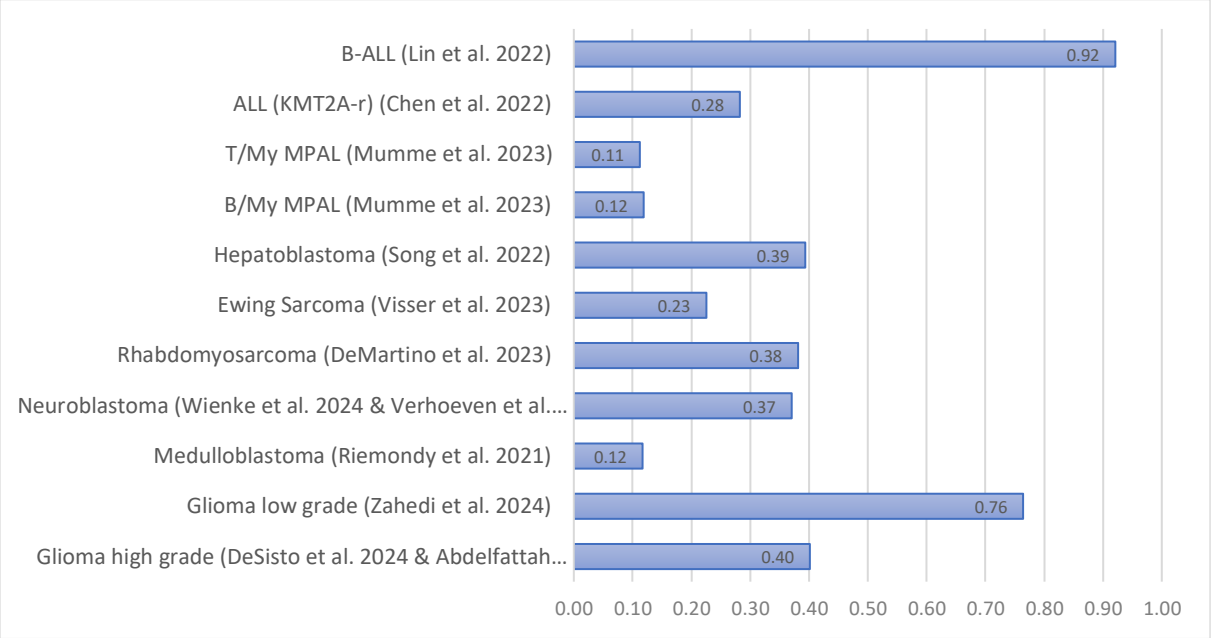

Figure 2: Total immune cell infiltration. Total immune cell fraction of total number of cells in patient samples per cancer subtype.

Creating an immune cell subset specific atlas for pediatric solid tumors

Pediatric solid tumors in specific have shown poor response to immunotherapeutic applications[19]. The immunological and pathophysiological barriers hampering this response therefore deserve additional thorough investigation to improve treatment outcome in children with solid tumors. To hone in on the distinct immune landscape of pediatric solid tumors, we aimed to unravel the exact presence of specific immune cell subsets that are at play in the immune TME of our retrieved publications. Before composing the tumor immune landscape across all obtained studies, we first merged the immune cell subset specific fractions of the total immune cell population within the TME among publications covering the same cancer subtype.

The Immune landscape of neuroblastoma

Neuroblastoma is the most common extracranial pediatric solid tumor, mainly affecting children under the age of 5. Tumors associated with this disease originate in the sympathetic nervous system, with common site of development being the adrenal glands, but furthermore including nerve tissue along the spine, chest, abdomen and pelvis as well[36]. To unravel the composition of the neuroblastoma immune TME based on our obtained publications, we first had to merge the scRNA-seq results from Wienke et al. and Verhoeven et al. We synthesized the fractions of the total immune cell population in the neuroblastoma immune TME for each specific immune cell subset as earlier described in the synthesis method. Figure 3 shows the immune cell subset specific fractions of the total immune cell population in the neuroblastoma TME for Wienke et al. (left column), Verhoeven et al. (middle column) and our merged results (right column). Both studies show roughly similar fraction values for each immune cell subset, with more than half of the total immune cell population being accounted for by T cells. In the study by Wienke et al. the total myeloid population was the second biggest fraction of total immune cells in the TME, while the study by Verhoeven et al. found the proportion of both the T helper and T effector cells to be bigger than the overall myeloid population. In both studies, regulatory T cells

(T reg cell) comprised the smallest immune cell subset population within the immune TME of neuroblastoma. The merged results show the synthesized data, displaying our obtained immune cell landscape of Neuroblastoma by scRNA-seq data (figure 3). Again, the proportion of the total amount of T cells is highest among all immune cell subsets in the immune TME. Both studies included a mix of patients in their cohort regarding previously obtained treatment. The studies however differed in the use of platform technology for scRNA-seq. Regarding data analysis for cell type assessment, Verhoeven et al. used the R package Conos, while Wienke et al. used the R packages SingleR and Seurat. Furthermore, Wienke et al. performed gene set enrichment analysis to compare their identified immune cell subset populations to the immune cell populations identified in the study of Verhoeven et al. and showed strong established similarities[31].

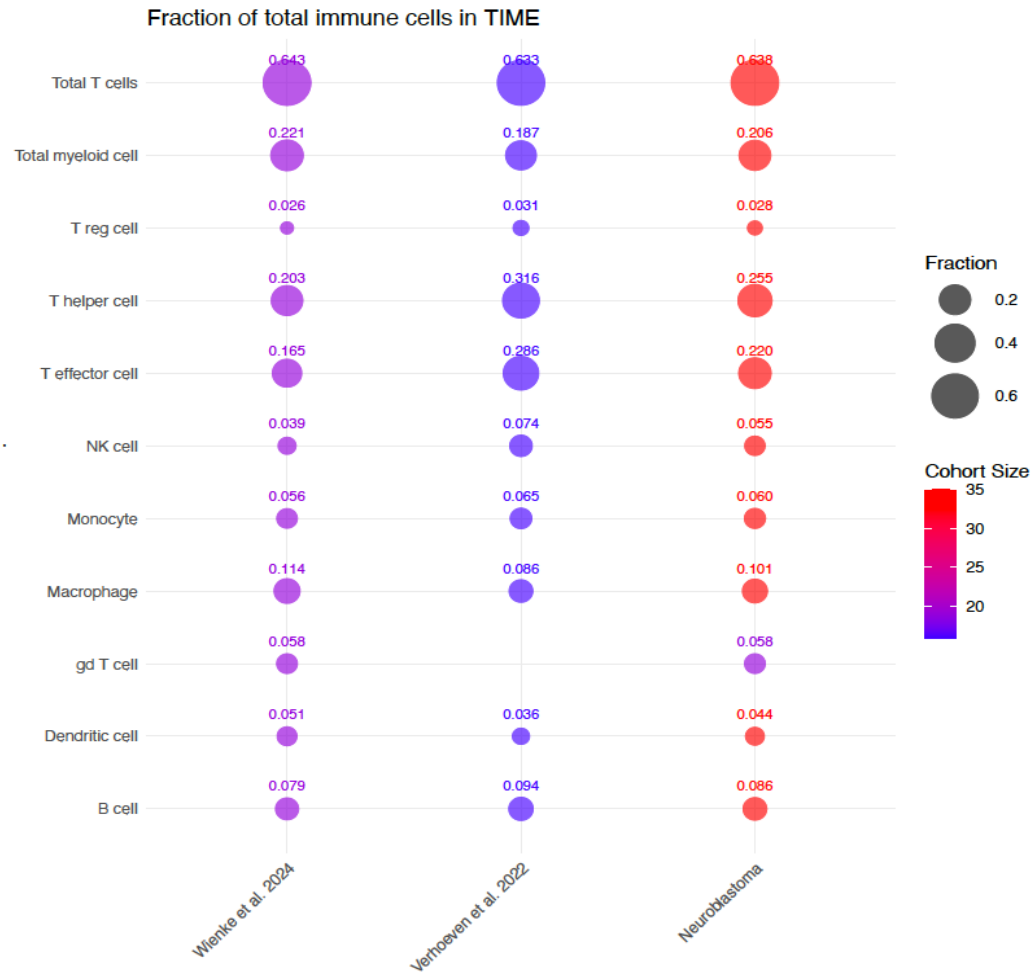

Figure 3: Dotplot showing the fraction size of distinct immune cell subsets in the total immune cell population of the neuroblastoma TME. The column on the left shows scRNA-seq results by Wienke et al. The column in the middle shows scRNA-seq results by Verhoeven et al. The column on the right shows the synthesized data of both studies (Wienke et al. and Verhoeven et al.).

The immune landscape of high-grade gliomas

High-grade gliomas are aggressive brain tumors with poor prognosis in pediatric patients. They can be primarily divided in grade III and grade IV tumors, and include subtypes like glioblastoma, anaplastic astrocytoma, diffuse midline glioma and diffuse intrinsic pontine glioma. These tumors originate from supportive glial cells which make up the supportive tissue of the brain[37]. To establish an understanding of the pediatric tumor immune landscape of high-grade gliomas based on scRNA-seq data, we merged

the scRNA-seq results from Abdelfattah et al. and DeSisto et al. We synthesized the fractions of the total immune cell population in the high-grade glioma TME for each specific immune cell subset. Figure 4 shows the immune cell subset specific fractions of the total immune cell population in the high-grade glioma TME for Abdelfattah et al. (left column), DeSisto et al. (middle column) and our merged results (right column). Both studies show similar fraction sizes for total T cell populations and total myeloid populations, with the total myeloid populations encompassing the greatest proportion in the total immune cell population in both studies. However, a difference in microglia fraction size is important to note. This immune cell subset fraction in Abdelfattah et al. their study is roughly twice the size of the fraction of microglia in the study by DeSisto et al. Furthermore, DeSisto et al did not cluster for macrophages and T reg cells, where Abdelfattah et al. did. On the contrary, DeSisto et al did cluster for monocytes, while Abdelfattah et al. did not. Although DeSisto et al. did not cluster for general macrophages, they did identify cells with an M2 like macrophage signature. The merged results show the synthesized data, displaying our obtained immune cell landscape of the TME in high-grade gliomas by scRNA-seq data (figure 4). This landscape is characterized by a relatively high myeloid infiltration as opposed to a smaller proportion of overall T cells in the total number of immune cells in the TME of gliomas. Among these studies, the risk of bias is important to consider. Here fore we assessed cohort group characteristics, clustering analysis method, treatment regimen and technological variability. Between the studies of Abdelfattah et al. and DeSisto et al. there is a possibility that cohort group characteristics could have impacted the merged results. Reason for this is that the study of Abdelfattah et al. only included one patient diagnosed with glioblastoma, while the cohort of DeSisto et al. contained heterogeneity in included high-grade glioma subtypes, consisting of patients diagnosed with glioblastoma, diffuse midline glioma, diffuse hemispheric glioma and radiation induced high grade glioma. Also, both studies did not elaborate on treatment regimen of their included patients, which may lead to unknown variability, potentially affecting results. Regarding the method used for single cell annotation, DeSisto et al. used the R package Azimuth to assess single cell identity, where Abdelfattah et al. used Seurat and SingleR. Both studies utilized the 10x genomics platform.

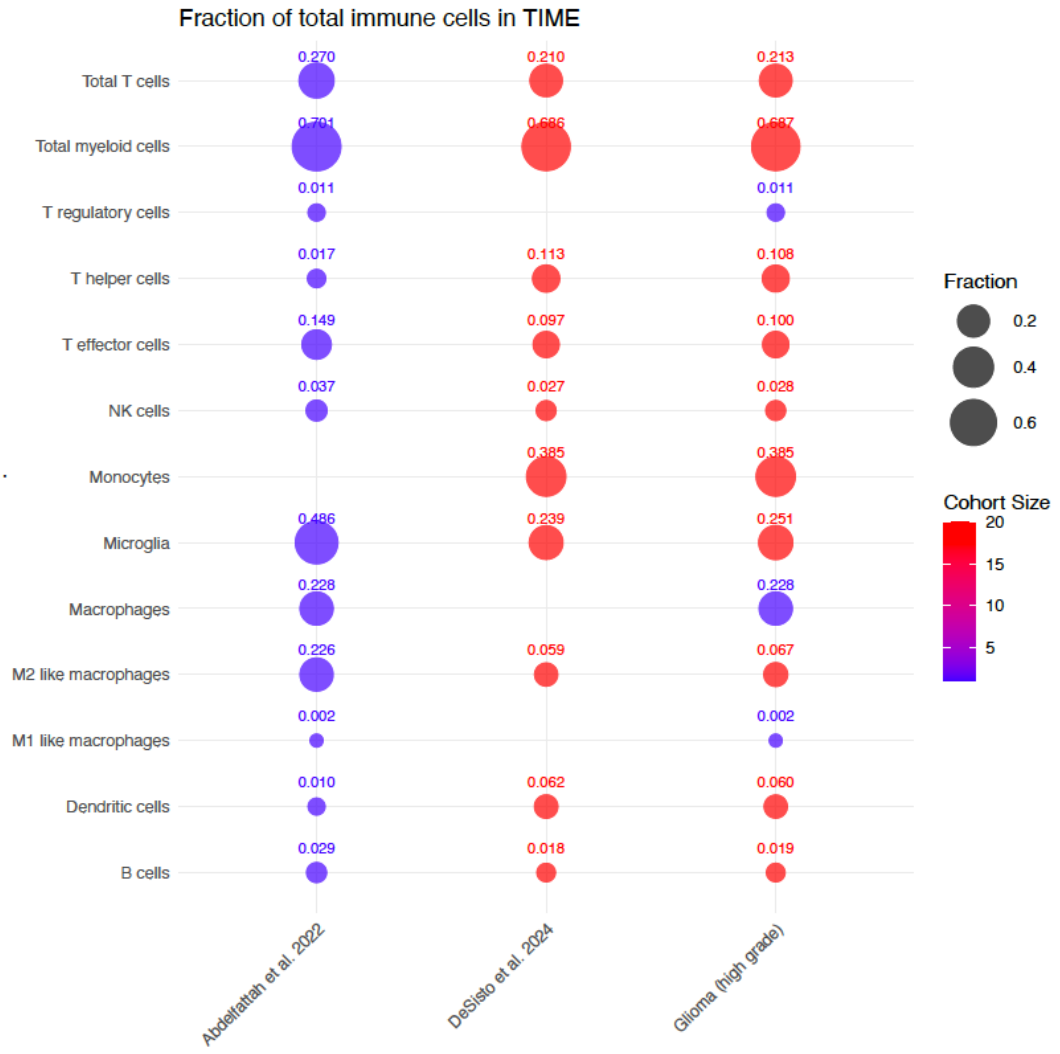

Figure 4: Dotplot showing the fraction size of distinct immune cell subsets in the total immune cell population of the high-grade glioma TME. The column on the left shows scRNA-seq results by Abdelfattah et al. The column in the middle shows scRNA-seq results by DeSisto et al. The column on the right shows the synthesized data of both studies (Abdelfattah et al and DeSisto et al.).

Other included solid tumors

Besides neuroblastoma and high-grade glioma, our obtained publications comprised immune cell data on several other solid tumors, including low grade glioma, medulloblastoma, hepatoblastoma, rhabdomyosarcoma and Ewing sarcoma. For these tumor types we obtained only one study per tumor type, which meant that there was no need for the merging of immune cell subset data from multiple studies to form integrated results. Pediatric low-grade glioma are the most common brain tumors in children and have a relatively good prognosis compared to high-grade gliomas. These tumors also commonly arise from glial cells that support the structure and function of the central nervous system[38]. Medulloblastoma is another common pediatric brain tumor. These tumors originate from embryonic cells in the posterior fossa[39]. Pediatric hepatoblastoma is the most common cancer in young children, usually occurring in the first 3 years of life. It is an embryonic tumor originating mainly from hepatocellular precursor cells and has a generally favorable prognosis[40]. Lastly, we obtained two studies on sarcomas, including rhabdomyosarcoma and Ewing sarcoma. The first is the most common soft tissue sarcoma in children. It's a very heterogenous disease and it arises from skeletal muscle cells[41].

Ewing sarcoma is a rare, but highly aggressive solid tumor primarily affecting children and adolescents. It mainly arises in the long bones of the body, but it can also occur in soft tissue[34].

Utilizing the pediatric solid tumor immune atlas to fit tailored immunotherapy strategies

Merging the scRNA-seq results from the obtained pediatric solid tumor studies together with our synthesized scRNA-seq results of neuroblastoma and high-grade glioma, enables us to create an immune atlas for all distinct pediatric solid tumors obtained for this analysis. Within this atlas we can compare immune cell subset specific fractions within the total immune cell population of the TME's across various pediatric tumors (figure 5).

To globally compare the signature of the immune landscape among distinct pediatric solid tumors, we can study the fractions of total T cells and total myeloid cells in the total immune cell populations of the TME. Strikingly, in the immune cell population of all solid brain tumors, myeloid cells are predominantly present with exact fractions of 0,763, 0,687 and 0,542 for low-grade glioma, high-grade glioma and medulloblastoma respectively. Contrary, both sarcomas are characterized by a predominant total T cell population, with exact fractions of 0,573 and 0,465 for rhabdomyosarcoma and Ewing sarcoma respectively. Just as seen for the sarcomas, the TME of neuroblastoma is also characterized by a relatively high T cell infiltration with a fraction of 0,638, compared to the influx of total myeloid cells in its TME, with exact fraction being 0,206. Furthermore, we note that the immune TME of hepatoblastoma is characterized by a large population of myeloid cells, with nearly 50% of this myeloid population consisting of macrophages. Compared to all other solid tumors, the proportion of macrophages in the total immune cell population in the TME of hepatoblastoma is especially high. Furthermore, B cell tumor infiltration within both high- and low-grade gliomas was relatively low, 0,019 and 0,009 respectively, compared to all other solid tumors. To be more precise, especially Ewing sarcoma, rhabdomyosarcoma and neuroblastoma showed a lot higher B cell fractions in the total immune TME, 0,068, 0,073 and 0,086 respectively. Although our obtained data gives insight in specific immune cell fractions across all cancer types for certain immune cell populations, data on several immune cell subsets is only limited to a minimal number of cancer subtypes. E.g., only one study (high-grade glioma) obtained exact fraction data on M1 like macrophages. Moreover, data on M2 like macrophage fractions was only obtainable from publications on high-grade gliomas and medulloblastoma. Furthermore, there was also a shortage on obtainable gd T cell data, with exact immune cell fractions only available for publications by Wienke et al. and DeMartino et al for this specific immune cell subset. Scarcity regarding obtainable exact data for several specific immune cell subsets has resulted in blanks within our pediatric solid tumor immune cell atlas, preventing the comparison of these immune cell subsets across all included pediatric solid tumors, and therefore limiting the use of our atlas for applications that are related to these specific immune cells.

It's important to note that the scRNA-seq data in the acquired studies for the assembly of the immune atlas were not all obtained under homologous circumstances, and therefore might contain risks of bias. As seen in table 1, treatment regimen before sampling varied widely in cohort groups across all studies, potentially influencing their TME immune data. Moreover, not all publications used the same markers for identifying and annotating cell clusters, leaving the possibility for inconsistency regarding immune cell identification across studies, potentially affecting TME immune composition results.

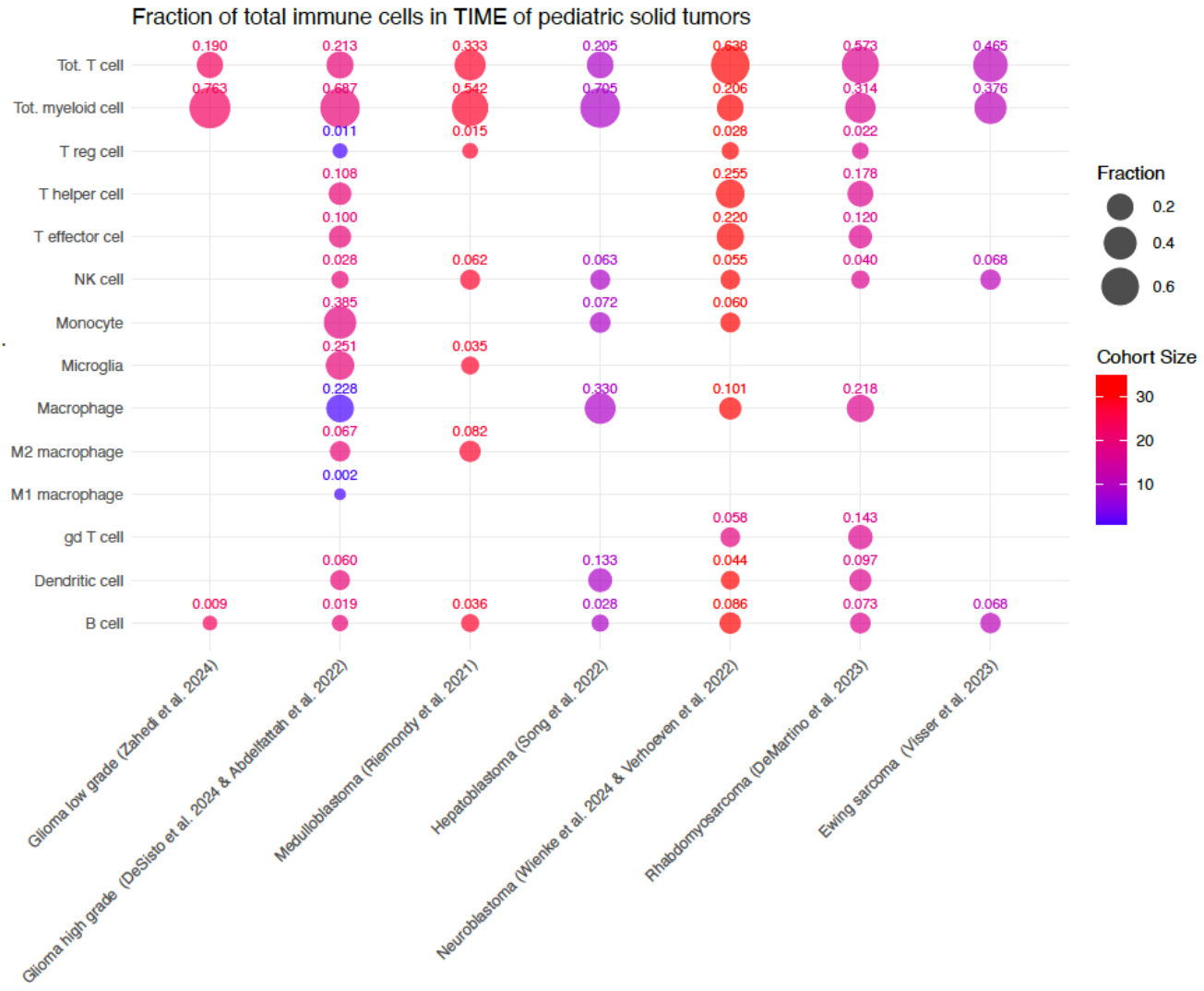

Figure 5: Dotplot showing the fraction size of distinct immune cell subsets in the total immune cell population of the TME across various pediatric solid tumors. This forms the construction of an immune atlas for pediatric solid tumors.

We ought our constructed immune atlas to guide future immunotherapeutic strategies for pediatric solid tumors, as current effective therapies are lacking for this group of patients. Analysis of distinct immune cell subset prevalence and their expected role within the TME of our included pediatric cancer types can indicate specific potential tumor responsiveness to immunotherapies like immune checkpoint inhibitors, adoptive cell therapy and myeloid targeted therapies. These therapies generally exert their effect by strengthening an existing immune response or restoring a suppressed immune response, both for which antigen presentation is of critical importance. As a consequence of a low mutational burden and a subsequent reduced neoantigen presentation in pediatric solid tumors however, these cancer types have low immunogenicity and antigenicity for such immunotherapies, presumably causing the lack of effective immunotherapeutic agents in the clinic to treat pediatric cancers[42].

To overcome this hurdle and develop tailored immunotherapeutic strategies in pediatric solid cancers, we therefore suggest combination therapies that integrate the use of oncolytic virus (OV) therapy with cancer subtype specific immunotherapies like checkpoint inhibitors, adoptive cell therapy or myeloid targeted therapy based on our pediatric cancer immune atlas. Laying at the base of the rationale for this is the ability of OV's to create a proinflammatory TME and increase the immunogenicity of the

tumor, thereby setting the stage for other immunotherapies[43]. OV's exert their antitumor effect in two ways. First, they can specifically target cancer cells, where they replicate and kill tumors cells by lysing them. Secondly, the lysis of tumor cells results in the release of tumor associated antigens in the TME, stimulating a robust immune response[44]. Via this second mechanism OV's may help compensate for the low neoantigen presentation present in pediatric tumors, and the resulting inductions of proinflammatory conditions in the TME can help to switch tumors from typically immunologically "cold" to "hot"[45]. OV's can be wild-type viruses with natural tropism for tumor cells, or genetically modified viruses to achieve tumor-specific virulence[43]. Moreover, OV's can be genetically designed to express therapeutic genes that could exert profound anti-tumor responses via different therapeutic mechanisms[46].

Given the relatively high prevalence of myeloid cells in the TME of pediatric gliomas (Low-grade glioma, high-grade glioma) based on our atlas, combined with the proposed believe that pediatric gliomas are characterized by an immunosuppressive TME, we believe it to be highly strategic to focus on a combination therapy consisting of an OV therapy combined with a myeloid targeted therapy to battle this type of cancer.[47] Using molecular therapies that reprogram tumor associate macrophages from a pro-tumoral phenotype to a more anti-tumoral phenotype, like CSF-1R inhibitors and PI3Ky inhibitors, mitigates myeloid-driven suppression, and thereby indirectly enhances the host' innate and T cell immune response[47,48]. Together, this presents a multifaceted approach to enhance immune anti-tumor response.

Medulloblastoma also shows a relatively dominant presence of myeloid cells in its immune TME, but it has a higher relative total T cell ratio than gliomas (figure 5). It furthermore differs from pediatric gliomas by having an even lower total immune cell infiltration, which was shown both in our own immune atlas and in earlier publications (figure 2)[49,50]. The immune cells that however do infiltrate, putatively have an immunosuppressive signature[51]. For this tumor subtype, we therefore suggest utilizing a combination therapy of OV therapy genetically modified to express genes with myeloid targeted therapeutic effects, together with immune checkpoint therapy, potentially resulting in a synergistic working mechanism, where the OV's with integrated myeloid targeted therapy elevate T cell influx, and immune checkpoint therapy unleashes the brakes on these T cells. Such modified OV's are currently being intensively investigated[52,53].

Compared to all pediatric solid tumors included in this review, hepatoblastoma shows a relatively high influx of total immune cells in the TME (figure 2). Furthermore, our atlas shows a relatively high dendritic cell proportion and a prevalent population of NK cells for hepatoblastoma. This prevalence of NK cells is further confirmed by Guo et al., who additionally found that KIR2DL is significantly upregulated in hepatoblastoma patients[54]. Tumor cells can bind to this inhibitory receptor on NK cells, inhibiting the cytotoxicity of NK cells, resulting in immune evasion in hepatoblastoma. Considering these characteristics of hepatoblastoma, we propose a combination therapy targeting these crucial findings. This combination includes the use of a genetically modified OV to express GM-CSF, combined with the administration of a KIR2DL inhibitor. This suggestively results in a working mechanism where OV's promote cell lysis, initiating the release of tumor associated antigens, while simultaneously expressing GM-CSF. These two factors together enhance the recruitment and activation of an already relatively large dendritic cell population, which on its turn presents tumor antigens to T cells and stimulate cytotoxic activity of NK cells[55]. This effect on an already prevalent NK cell population in hepatoblastoma is then supposed to be further potentiated by the administration of a KIR2DL inhibitor. A similar modified OV strategy has shown promising results in osteosarcoma mouse models earlier[52].

Our atlas shows moderate prevalence of effector T cells and NK cells in the total immune population in the TME of Neuroblastoma, which is confirmed by earlier publications as well[56,57]. Wienke et al. showed the presence of an immunosuppressive TME in neuroblastoma, characterized by immunosuppressive myeloid population and dysfunctional profiles of T and NK cells through increased expression of PD-1

and TIGIT, respectively[31]. With Wienke et al. already identifying and demonstrating TIGIT and PD-L1 to be promising immune checkpoint inhibition targets to treat neuroblastoma, our suggested atlas guided treatment strategy is in line with their findings. We propose a combination therapy that combines OV therapy with the administration of immune checkpoint inhibitors targeting TIGIT and PD-L1, resulting in the following supposed effect. Neuroblastoma specific OV's can selectively infect and lyse tumor cells, resulting in the release of tumor associated antigens and tumor cell death. This induces proinflammatory conditions in the TME and attracts immune cells to the tumor site, including concurrently activated NK cells[45]. The simultaneous administration of anti-PD-L1 and anti-TIGIT therapeutic molecules will synergize with the OV effect by directly mitigating the dysfunctional signature of the attracted T and NK cells in the TME, allowing for efficient killing of neuroblastoma cells.

By evaluating the immune landscape of rhabdomyosarcoma in our pediatric immune atlas, we can observe that effector T cells and NK cells are moderately prevalent in the immune TME of rhabdomyosarcoma. DeMartino et al. further determined that TIGIT was overexpressed on CD8+ T cells, giving rise to an immune dysfunctional phenotype. Moreover, the myeloid cell population in rhabdomyosarcoma is characterized by M2 polarized macrophages, indicating an immunosuppressive TME[33]. The TME of rhabdomyosarcoma is furthermore also composed of a considerable dendritic cell population (figure 5). This given immune landscape thus poses multi-targetable options accompanied by the prevalence of multiple effector cells. To treat rhabdomyosarcoma, we propose a combination therapy utilizing an oncolytic virus modified to express GM-CSF, combined with the administration of an anti-TIGIT immune checkpoint inhibitor to synergistically enhance anti-tumor efficacy while modulating the TME to a more inflammatory character. OV's are capable to specifically target tumor cells and lyse them, causing the release of tumor associated antigens. While concomitantly expressing GM-CSF, we suggest that this results in recruitment of NK cells, the polarization of M2 macrophages to M1 macrophages, and the recruitment and differentiation of dendritic cells to present antigens (that are released by OV cell lysis) for the initiation of a specific T-cell response[58,59 60]. By additional administration of anti-TIGIT immune checkpoint inhibitors, CD8+ T cells and NK cells are stripped of their dysfunctional character to synergistically enhance anti-tumor response.

According to our obtained data, Ewing sarcoma displays a relatively low overall influx of immune cells in the TME (figure 2). This has been verified by earlier publications as well[61]. Furthermore, there is a relatively high NK prevalence in Ewing sarcoma (figure 5), and the myeloid population in the TME of Ewing sarcoma is predominantly characterized by the immunosuppressive M2-like phenotype[34]. Additionally, high immunogenic natural T cell epitopes have not been identified for Ewing sarcoma[62]. Given this immune signature of the Ewing sarcoma TME, focusing on a strategy that maximizes the cytotoxic potential of NK cells, combined with the use of an OV, provides a promising avenue for effective treatment of Ewing sarcoma. We therefore suggest the application of an OV which is genetically modified to express CXCL10. Additional to the induction of a pro-inflammatory TME by OV's, CXCL10 creates a strong chemotactic gradient within the TME which directs NK cells, but also other cytotoxic lymphocytes, precisely to the tumor location and enhances their activation. The potential of the use of CXCL10 is further underscored by publications showing a positive correlation of the presence of this chemokine with higher tumor infiltrating lymphocytes and a consecutive survival benefit, and by studies revealing the pathophysiologic absence of CXCL10 in the TME of Ewing sarcoma[61,63]. Also, the use of an CXCL10 modified OV has shown promising results in a colon cancer mouse model recently[64]. Whether NK cells in Ewing sarcoma display dysfunctional immune profiles has been less investigated. Nevertheless, Visser et al. demonstrate an increased expression of HAVCR2 by NK cells[34]. If additional research confirms this dysfunctional NK cell profile in Ewing sarcoma, we propose the subsequent administration of anti-HAVCR2 immune checkpoint inhibitors in addition to our suggested CXCL10 OV therapy, to induce a synergistic effect on the antitumor response by NK cells in Ewing sarcoma.

Ultimately, we proposed tumor type specific rationales for immunotherapeutic interventions based on our pediatric solid tumor immune atlas, overlapping pediatric cancer hallmarks, and molecular signatures of solid tumor immune cell populations based on additional literature. It's important to note that the proposal of new therapeutic strategies for pediatric solid cancers demands more thorough investigation than demonstrated in our provided rationales above. Providing in dept motives for the choice of virus sub-types and a specific combinatory immunotherapeutic strategy does not fully fit the scope of this review. The rationales for the proposed immunotherapeutic interventions mentioned above merely demonstrate the capacity of our pediatric immune atlas to guide immunotherapeutic choice for pediatric solid tumor treatment.

Discussion

The lack of effective immunotherapeutic strategies in clinical use to treat pediatric cancer types underscores the need for the development of immunotherapies that are specifically tailored to the unique immune TME of pediatric cancers to ensure effective treatment. To attain such therapies, an accurate characterization of the cellular and molecular components at play in the immune TME of distinct pediatric cancer subtypes is crucial. Here, we performed a meta-analysis to contribute to this goal. By utilizing a systemic search strategy and following the PRISMA guideline standards, we have tried to identify all available publications which analyzed the immune profile of the TME of pediatric cancer types using scRNA-seq. From these publications we assembled all available total immune cell fraction data for all obtained cancer subtypes. Furthermore, we assembled all available immune cell subset specific fraction data of all obtained solid tumor types. By integrating the latter information, we constructed an immune atlas for distinct pediatric solid tumors obtained for this analysis, which we can utilize to compare immune cell subset specific fractions within the total immune cell population of the TME's across various pediatric tumors. This ultimately served the goal to guide future treatment responsiveness and novel immunotherapy development in pediatric oncology.

According to our obtained scRNA-seq data, the total immune cell infiltration, here depicted by the fraction of total immune cells as part of the total numbers of cells in the patient samples, varies tremendously across all obtained pediatric cancer types. Relatively low total immune cell fractions were observed for T/My MPAL, B/My MPAL and medulloblastoma. Moreover, high-grade glioma, neuroblastoma, hepatoblastoma, Ewing sarcoma, ALL (KMT2A-r aberration) and rhabdomyosarcoma were found to be characterized by intermediate immune cell infiltration, while B-ALL and low-grade glioma were characterized by a relatively high infiltration of immune cells. Regarding immune cell subset prevalence in the total immune cell populations of pediatric solid tumors, the most significant findings include predominantly prevalent total myeloid cell populations in low-grade glioma, high grade glioma, medulloblastoma and hepatoblastoma, while neuroblastoma, rhabdomyosarcoma end Ewing sarcoma are characterized by relatively high proportions of total T cells.

Even though the immune landscape of pediatric solid tumors has generally been poorly investigated, it's important to set our results side by side to earlier findings. Our results show variable outcomes regarding immune infiltrate expectations based on earlier research for the included tumor types. Our obtained glioma (high-grade and low-grade) data largely corresponded with prior research, demonstrating that low-grade gliomas are characterized by a larger total T cell infiltration compared to high-grade gliomas[65]. This data was based on multiplex immunohistochemistry (IHC), machine learning and single cell mass cytometry. Moreover, other studies also verified a more cold TME signature for high-grade gliomas compared to low-grade gliomas, with relatively large proportions of myeloid cells for both types of gliomas[66]. In line with our findings, earlier research also demonstrated a far lower degree of immune infiltration in medulloblastoma as compared to other pediatric brain tumors like low-grade and high-grade gliomas[67]. Our obtained immune cell data on the TME of medulloblastoma

furthermore also confirms the general point of view that most pediatric brain tumors are signatured by a relatively high myeloid cell proportion and a relatively lower total T cell influx[68]. For our hepatoblastoma results, we demonstrate a relatively high immune infiltration compared to other pediatric solid tumors, just as generally thought in previous research[26]. Moreover, in accordance with earlier publications, we also show a substantial NK cell population[68,69]. There are however discrepancies regarding the myeloid to lymphocyte ratio in hepatoblastoma. Using scRNA-seq, Wang et al. show that myeloid cells account for the largest proportion of immune cells in hepatoblastoma followed by lymphocytes, which is in accordance with our own results[70]. On the contrary, a recent gene set enrichment analysis study on bulk RNA-seq data by Liu et al. demonstrate a slightly more prominent presence of total T cells compared to myeloid cells. Additionally, they also show low numbers of macrophages and high numbers of monocytes, while our findings show opposite results. Previous research on neuroblastoma shows that its TME is heterogeneous and immune composition of neuroblastoma differs per subtype and classification. Slight discrepancies between immune cell subsets and a lack of study into myeloid cell population are evident for Neuroblastoma as well[68]. Even though neuroblastoma is historically considered to be a cold tumor, bulk RNA-seq studies show residence of multiple T cell sub populations, like Treg cells, T effector cells, T helper cells and gdT cells, and NK cells[71]. This is in accordance with our results. Prevalence of T cells was also confirmed by an IHC study[72]. In contrast with our findings, earlier research report a rare or mostly absent B cell population in neuroblastoma[57]. Moreover, a deconvolution study on bulk RNA-seq data by Zhong et al. demonstrated that at least half of all cells in the neuroblastoma TME consisted of immune cells, which was divided in approximately 50% myeloid cells, and 50% lymphoid cells[73]. Our results on the other hand diverge from these findings, by displaying a total immune cell infiltration of 37%, which is composed of predominantly T cells (57%) and fewer myeloid cells (31%). For rhabdomyosarcoma, previous research based on IHC and digital pathology analysis suggested an immune TME where T cells and NK cells were scarcely prevalent as compared to a relatively more dominant myeloid population[74–76]. Our scRNA-seq results however contradict this suggestion by displaying higher numbers of total T cells than total myeloid cells. For Ewing sarcoma, our total immune cell infiltration results for are relatively low, which correlates with the general view of the presumed cold TME in Ewing sarcoma[77]. However, a previous IHC study determined a dominant presence of myeloid cells and a scarce prevalence of T cells in the total immune cell population of the Ewing sarcoma TME[8]. Besides, a gene expression microarray analysis by Stahl et al. also found myeloid to be abundantly present, with percentages of macrophages in the total immune infiltrating cell population being 43%, and a lower T cell influx, with percentages of T cells in the total immune infiltrating cell population being 23%[78]. Both these studies thus oppose our results regarding total T cell to myeloid cell ratios in Ewing sarcoma.

There could be various possible causes for the differences in immune cell fraction data obtained for the same cancer subtype regarding the comparison of our own results with earlier publications. Differences in study design for the inclusion of patients can cause heterogeneity in treatment regimen, age, gender and subtype, all possibly affecting immune cell fraction outcome. Moreover, the use of a different immune cell detection technique, like scRNA-seq, IHC, or bulk RNA-sequencing, can greatly influence immune cell recognition, and thereby fraction outcomes. ScRNA-seq can identify diverse cell types in a very specific way with a high resolution, however it also requires dissociation of the tumor into single cells, possibly affecting viability and therefore the detection of certain cell types[79]. Concerning bulk RNA-sequencing, this technique is limited to the detection of average prevalent immune signatures and a broad evaluation of the immune landscape of tumor samples, hereby lacking the cellular resolution to identify specific immune cell subsets[20,79]. Studies using IHC for immune cell detection on the other hand might introduce bias through the limitation for the allowance of broad marker detection. Furthermore, it is also restricted by difficulties in the quantitative analysis of immune cells compared to RNA-based detection methods[80].

While our constructed pediatric immune atlas for solid tumors forms a great framework to identify and quantify the specific immune cell subsets at play in the TME of specific pediatric cancers, it currently

holds its limitations for the implementation to guide future immunotherapeutic responsiveness and immunotherapeutic strategy development for pediatric cancer patients. By focusing exclusively on the types and fractions of immune cell subsets, without considering the functional states of these immune cells, this may overlook critical aspects of cancer type specific TME immune signatures which influence the progression and treatment outcomes of pediatric cancers. Immune cells in the TME can exhibit a range of dysfunctional states. T cells can exert exhaustive and anergic states, contributing to immune evasion of tumor cells by progressive loss of effector functions[19]. Similarly, NK cells can also be characterized by dysfunctional states, associated with reduces cytotoxic activity. Furthermore, macrophages and myeloid-derived suppressor cells in the TME can be polarized to an anti-inflammatory (pro-tumoral) state, suppressing adaptive and innate immune responses[12]. These states are often characterized by distinct gene expression profiles which cannot be discerned merely by quantifying cell types and are not integrated in our immune atlas. Since therapeutic strategies are increasingly relying on leveraging these functional states of immune cells, the absence of information on functional cell states limits the potential clinical utility of our pediatric immune atlas[12]. Thus, our created pediatric immune atlas as presented in its current form (figure 5), while valuable, should be interpreted with vigilance if used to directly guide immunotherapeutic decision-making. This meta-analysis should therefore be seen as a foundational step towards a broader understanding of the immunological character of TME's across pediatric solid tumors. To improve this understanding and increase the clinically applicable potential of our immune atlas, future studies should aim to incorporate the functional states of specific immune cells into the current immune atlas.

To add to that, our analysis only comprises the pediatric cancer types that were included based on our inclusion criteria. The span of our immune atlas is therefore limited by the choice of our eligibility criteria. Additionally, our immune atlas also shows blanks, which result from a lack in published numerical data for certain immune cell subsets. These restrictions are a result of limited exact data availability in most of recently published studies investigating the immune landscape of pediatric cancer types. As noticed during the selection of eligible publications, we screened a lot (n=24) of studies that were only not found to be eligible because they solely displayed their immune cell results in figures or graphs, from which no exact data was legible. As a result from this, many publications containing valuable information on the immune landscape of certain pediatric cancers were excluded because of the inability to quantify and synthesize their results for the integration in a meta-analysis. We, therefore, advocate for the standardization of exact immune cell data presentation in studies examining the immune landscape of pediatric tumors. This standardization is crucial for enabling a thorough comprehensive analysis on the immune TME across various pediatric cancer types in future studies.

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Supplementary data

Supplementary data 1; Search Terms:

((("single cell"[All Fields] OR "single-cell*"[All Fields]) AND ("sequencing*"[All Fields] OR "RNA sequencing*"[All Fields] OR "RNA-seq*"[All Fields] OR "genome*"[All Fields] OR "genomics*"[All Fields] OR "transcriptome*"[All Fields] OR "transcriptomics*"[All Fields])) OR "scRNA-seq*"[All Fields] OR "scRNAseq*"[All Fields] OR "scRNA seq*"[All Fields] OR "sc-RNAseq*"[All Fields] OR "sc RNAseq*"[All Fields]) AND ("immune"[All Fields]) AND ("paediatric*"[All Fields] OR "pediatric*"[All Fields] OR "child*"[All Fields] OR "infants*"[All Fields] OR ("adults*"[All Fields] AND "young*"[All Fields])) AND ((("NK cell*"[All Fields]) OR "NK cells"[All Fields] OR "Natural killer cells"[All Fields] OR ("Natural killer cell"[All Fields]) OR ("CD56"[All Fields])) OR (("B cell*"[All Fields]) OR "B cells"[All Fields] OR "B lymphocyte"[All Fields] OR ("L26"[All Fields]) OR ("CD20"[All Fields]) OR ("CD19"[All Fields])) OR (("macrophage*"[All Fields]) OR "macrophages"[All Fields] OR ("CD68"[All Fields]) OR ("CD163"[All Fields]) OR ("TAM"[All Fields]) OR "tumor infiltrating macrophages"[All Fields] OR ("M1"[All Fields]) OR ("M2"[All Fields])) OR (("myeloid*"[All Fields]) OR "myeloid cells"[All Fields] OR ("CD33"[All Fields])) OR (("T cell*"[All Fields] OR "T cells"[All Fields] OR "T lymphocyte"[All Fields] OR "T regulatory"[All Fields] OR "regulatory T"[All Fields] OR "helper T cell"[All Fields] OR "cytotoxic T cell"[All Fields] OR "memory T cell"[All Fields] OR "killer T cell"[All Fields] OR "CD3"[All Fields] OR "CD4"[All Fields] OR "CD8"[All Fields] OR "FOXP3"[All Fields] OR "CD45"[All Fields] OR "CD25"[All Fields] OR "Th1"[All Fields] OR "Th2"[All Fields] OR "Th17"[All Fields] OR "Th0"[All Fields] OR "Treg"[All Fields] OR "TIL"[All Fields] OR "tumor-infiltrating lymphocytes"[All Fields] OR "gammadelta"[All Fields]))) AND ("neuroblastoma*"[All Fields] OR "rhabdomyosarcoma*"[All Fields] OR ("sarcoma"[All Fields] AND "synovial"[All Fields]) OR ("MPNST"[All Fields] OR "neurofibrosarcoma"[All Fields] OR ("malignant"[All Fields] AND "peripheral"[All Fields] AND "nerve"[All Fields] AND "sheath"[All Fields] AND ("tumour*"[All Fields] OR "tumor*"[All Fields]))) OR ("sarcoma*"[All Fields] AND "ewing*"[All Fields]) OR "osteosarcoma*"[All Fields] OR "ATRT"[All Fields] OR ("atypi*"[All Fields] AND "teratoid rhabdoid"[All Fields] AND ("cysts"[MeSH Terms] OR "cysts"[All Fields] OR "cyst"[All Fields] OR "neurofibroma"[MeSH Terms] OR "neurofibroma"[All Fields] OR "neurofibromas"[All Fields] OR "tumor*"[All Fields] OR "tumour*"[All Fields] OR "neoplasm*"[All Fields])) OR ("rhabdoid"[All Fields] AND ("tumour*"[All Fields] OR "tumor*"[All Fields])) OR ("wilm*"[All Fields] AND ("cyst*"[All Fields] OR "neurofibroma*"[All Fields] OR "tumor*"[All Fields] OR "tumour*"[All Fields] OR "neoplasm*"[All Fields])) OR "nephroblastoma*"[All Fields] OR "hepatoblastoma*"[All Fields] OR (("granuloma*"[All Fields] AND "plasma"[All Fields] AND "cell"[All Fields]) OR ("inflammatory"[All Fields] AND "myofibroblastic"[All Fields] AND "tumour"[All Fields])) OR ("extracranial*"[All Fields] AND ("germ cell tumour"[All Fields] OR "neoplasms, germ cell and embryonal"[MeSH Terms] OR ("neoplasms"[All Fields] AND "germ"[All Fields] AND "cell"[All Fields] AND "embryonal"[All Fields]) OR ("germ"[All Fields] AND "cell"[All Fields] AND "tumor"[All Fields]) OR "germinoma"[MeSH Terms] OR "germinoma"[All Fields] OR ("germ"[All Fields] AND "cell"[All Fields] AND "tumor"[All Fields]))) OR "retinoblastoma*"[All Fields] OR ("glioma"[MeSH Terms] OR "glioma*"[All Fields]) OR ("diffuse"[All Fields] AND "intrinsic"[All Fields] AND "pontine"[All Fields] AND "glioma"[All Fields]) OR "ependymoma*"[All Fields] OR "medulloblastoma*"[All Fields] OR " primitive neuroectodermal tumors*"[All Fields] OR "germ cell tumors*"[All Fields] OR "Langerhans cell histiocytosis*"[All Fields] OR "lymphoma*"[All Fields] OR "Hodgkin lymphoma*"[All Fields] OR "anaplastic large cell lymphoma*"[All Fields] OR "Primary mediastinal B-cell lymphoma*"[All Fields] OR "non Hodgkin*

lymphoma"[All Fields] OR "Burkitt lymphoma*"[All Fields] OR "Diffuse large B-cell lymphoma*"[All Fields] OR "T cell lymphoblastic lymphoma*"[All Fields] OR "B cell lymphoblastic lymphoma*"[All Fields]).*

Layman's summary

Cancer is still one of the leading causes of deaths worldwide, and the continuous battle to eradicate this disease is far from over. Cancer is characterized by cells within the human body which grow uncontrollably, thereby disrupting normal body functions. In the past decade, immunotherapy has emerged as a transformative approach in cancer treatment, showing potential in the treatment of different types of cancer. In oncology, immunotherapy comprises treatments that enhance patients their own immune system to recognize and destroy cancer cells more effectively. Even though effective in certain cancer types, its effectiveness is inconsistent and varies among patients. Treatment success is particularly challenging in pediatric cancer patients. Unlike adults, children with cancer often do not respond well to standard immunotherapies that rely on an already existing immune response to tumors. The reason for this is that pediatric tumors usually have fewer genetic changes or mutations compared to adult cancers, which makes them harder for the immune system to detect and subsequently kill. Another reason for the lacking clinical results in pediatric oncology of immunotherapy, is because the pathological background of pediatric cancers is extremely diverse and differs significantly from the more intensively studied adult cancer types. This diversity is reflected in a unique tumor immune microenvironment for individual pediatric cancer patients. This tumor immune microenvironment is the area surrounding the malignant cells of the tumor, which include various types of immune cells and other components. These surrounding cells and components cooperate with each other, and thereby influence how the tumor grows and spreads, but also how it responds to treatments like immunotherapy. Logically, a comprehensive understanding of the immune cells at play in this environment for different pediatric cancer types, is essential to develop tailored and effective immunotherapies. To add to the understanding of the tumor immune microenvironment, and thereby aid future immunotherapeutic development, we conducted a meta-analysis on existing studies that used single-cell RNA sequencing, an advanced technique to give insights into the types and amounts of immune cell subsets in the analyzed tumor samples, to investigate the immune landscape of pediatric cancers. For analysis and comparison of the immune landscape across pediatric cancer types we systemically reviewed and filtered existing literature on this subject to find publications that strictly met our chosen inclusion criteria. This resulted in the inclusion of 12 studies, from which we obtained data on the presence of specific immune cell subtypes within various pediatric cancer types. Using this data, we display the total immune cell infiltration in eleven pediatric cancer types based on our included studies. Eventually, we hone in on the immune landscape of pediatric solid tumors within our obtained publications, as they deserve more thorough investigation given the lack of currently available treatment options for these cancer types. Through the analysis and merging of our obtained data, we constructed a detailed map of the immune landscape in our included pediatric solid cancer types, which we call an immune atlas. This atlas offers insights in the immune cell subset populations that play a role in the tumor immune microenvironment of pediatric cancers, thereby forming a blueprint to guide future treatment responsiveness and immunotherapeutic strategy development in pediatric solid tumors. We subsequently demonstrate the potential use of our pediatric immune atlas by proposing immunotherapeutic treatment strategies guided by the content of our atlas. Our atlas however currently also holds its limitations for the direct use for clinical decision making, and this product of our meta-analysis should therefore be seen as a foundational step towards a broader understanding of the immunological character of the tumor microenvironment across pediatric solid tumors.