Nuclear Medicine and Biology 114–115 (2022) 58–64

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

# Biologicals as theranostic vehicles in paediatric oncology

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ARTICLE INFO

*Keywords:*
Theranostics
Nuclear imaging
Radionuclide therapy
Biologicals
Paediatric oncology

ABSTRACT

Biologicals, such as antibodies or antibody-fragments *e.g.* nanobodies, have changed the landscape of cancer therapy and can be used in combination with traditional cancer treatments. They have been demonstrated to be excellent vehicles for molecular imaging. Several biologicals for nuclear imaging of adult cancer may be used in combination with (nuclear) therapy. Though it's great potential, molecular imaging using biologicals is rarely applied in paediatric oncology. This paper describes the current status of biologicals as radiopharmaceuticals for childhood cancer. Furthermore, the importance and potential for developing additional biological theranostics as opportunity to image and treat childhood cancer is discussed.

## 1. Introduction

In the last decades, there has been an overall increase in European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA)-approved biologicals for, among others, cancer immunotherapy [1]. Biologicals, such as monoclonal antibodies (mAbs) and mAb-fragments, have a high specificity and affinity for their respective tumour specific markers. They consist of varying sizes from 15 to 130 kDa for mAb-fragments until 150 kDa for intact mAbs and are known for their high target accumulation, long retention and high tumour-to-background contrast. Subsequently, biologicals have been demonstrated in the past two decades to be excellent vehicles for molecular imaging and therapy.

For several adult cancers, the development and application of biological tracers for nuclear imaging and radionuclide therapy are well demonstrated and discussed in various recent reviews [2–7]. In addition, Pouget and colleagues listed the therapeutic radiopharmaceuticals that are currently in clinical trials for several adult cancer types [8]. Research towards paediatric oncology has only recently received attention, compared to adult cancer; hence, additional studies are necessary with respect to target validation and the development of radiopharmaceuticals. Challenges in theranostics development for childhood cancer are found in the small patient population that in addition covers a wide variety of different tumour types, *e.g.* neuro-oncology or solid tumours like neuroblastomas. Furthermore, the tumour biology of childhood cancers is distinct from adult cancers. Consequently only a few targets and theranostics can be translated from adult to childhood cancer. Therefore, theranostics development for childhood cancer is a field of research on its own where target discovery for theranostics development must be prioritized. As such there are individual biomarkers and target discovery programs which need to feed the general drug development as well as the nuclear theranostics development pipeline. To date, [$^{123/131}$I]mIBG is still the only theranostic available for routine clinical use to image and treat neuroblastoma tumours that express the norepinephrine transporter (NET). In addition, multiple studies are initiated with theranostics targeting SSTR-2A, CXCR4 and FAP-positive tumours, which were originally developed for adult cancers. Furthermore, the development of novel theranostics is still in its infancy.

This review evaluates the potential application of biologicals, especially focussing on mAb-fragments, for nuclear imaging and radionuclide therapy in paediatric oncology. First, the development and availability of biologicals will be discussed, specifically for paediatric cancers. Second, an overview of the current status of biologicals used in paediatric oncology as vehicles for molecular imaging and therapy will be provided. And finally, future perspectives are given for the development of new mAb-fragment theranostics for paediatric cancers, that

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https://doi.org/10.1016/j.nucmedbio.2022.09.001
Received 1 July 2021; Received in revised form 5 September 2022; Accepted 8 September 2022
Available online 14 September 2022

can improve the clinical outcome of children with cancer.

## 2. Biologicals as vehicles for molecular imaging and therapy

Biologicals, mAbs and mAb-fragments, are increasingly used in clinic to treat cancer [1]. At first, mAbs were generated as therapeutics for cancer, with the anti-tumour necrosis factor (TNF) mAb adalimumab being the first FDA-approved mAb-mediated therapy for psoriatic arthritis, ankylosing spondylitis and Crohn's disease [9]. In comparison to traditional drugs that are low molecular weight molecules, with a mass of around 500 Da, and peptides with a length of 10–20 amino acids that are not folded, mAbs and mAb-fragments have a higher affinity and specificity for the target. The first studied tumour targets for developing mAbs were cetuximab targeting epidermal growth factor receptor (EGFR), trastuzumab binding human epidermal growth factor receptor and rituximab for CD20 positive tumours [9,10]. Also, mAbs were generated targeting other tumour mechanisms, such as angiogenesis targeting vascular endothelial growth factor (VEGF) like bevacizumab. Later, mAbs against immune checkpoint inhibitors, such as ipilimumab for cytotoxic T lymphocyte antigen 4 (CTLA-4) and nivolumab, pembrolizumab, atezolizumab and durvalumab for PD-1/PD-L1 were developed for immunotherapy [11,12]. Additionally, mAbs can be used in combination with other cancer treatments. Because of their unfavourable physicochemical properties for therapeutic applications (*i.e.* high molecular weight precluding fast kinetics), there is now an increasing interest in developing low molecular weight mAb-fragments, such as nanobodies, because of their fast targeting, shorter blood circulation and fast excretion. The application and characteristics of mAbs and mAb-fragments are summarized in Fig. 1 and were reviewed in detail by Holliger and Hudson [4]. In general, mAb-fragments are excellent tumour targeting agents because of their pharmacokinetics and biodistribution characteristics.

Even though many biologicals, both mAbs and mAb-fragments, were developed in recent years for adult tumour therapy; for paediatric oncology, the use and application of biologicals is less developed. Currently, the only clinically approved mAb in standard care for paediatric solid tumours is dinutuximab (*i.e.* human/mouse chimeric antibody ch14.18), which is used as immunotherapy for GD2-positive neuroblastoma patients. Besides neuroblastoma, ongoing research on the development of new biologicals looks promising for several paediatric cancer types, including Diffuse Intrinsic Pontline Glioma (DIPG), Hodgkin Lymphoma (*e.g.* rituximab) and Acute Lymphocytic Leukemia (ALL) (*e.g.* bispecific mAb blinatumomab) [13,14]. In addition to these developments in generating novel biologicals, novel immunotherapeutic strategies for neuroblastoma were recently described, with the major challenge being the low immunogenicity of neuroblastoma [15]. In addition to cancer therapy, biologicals can be used in molecular imaging techniques, providing its importance in precision medicine.

Its effective and specific tumour targeting and increasing availability make biologicals based on mAb-fragments well suited as vehicles for molecular imaging and therapy, including fluorescence-guided surgery (FGS) and nuclear theranostics. Theranostics are defined by the combination of a diagnostic tracer and a therapeutic radiopharmaceutical, consisting of the same pharmaceutic moiety, radiolabelled with a diagnostic or therapeutic radionuclide. This provides a personalized medicine strategy (Fig. 2). Nuclear imaging is a tool that can be used to 1) diagnose patients, for disease staging, monitoring and tracking; 2) to select patients for treatments, such as immunotherapy, and 3) to determine the treatment strategy. Even though there are several immune checkpoint inhibitors approved by the FDA, it is not clear which

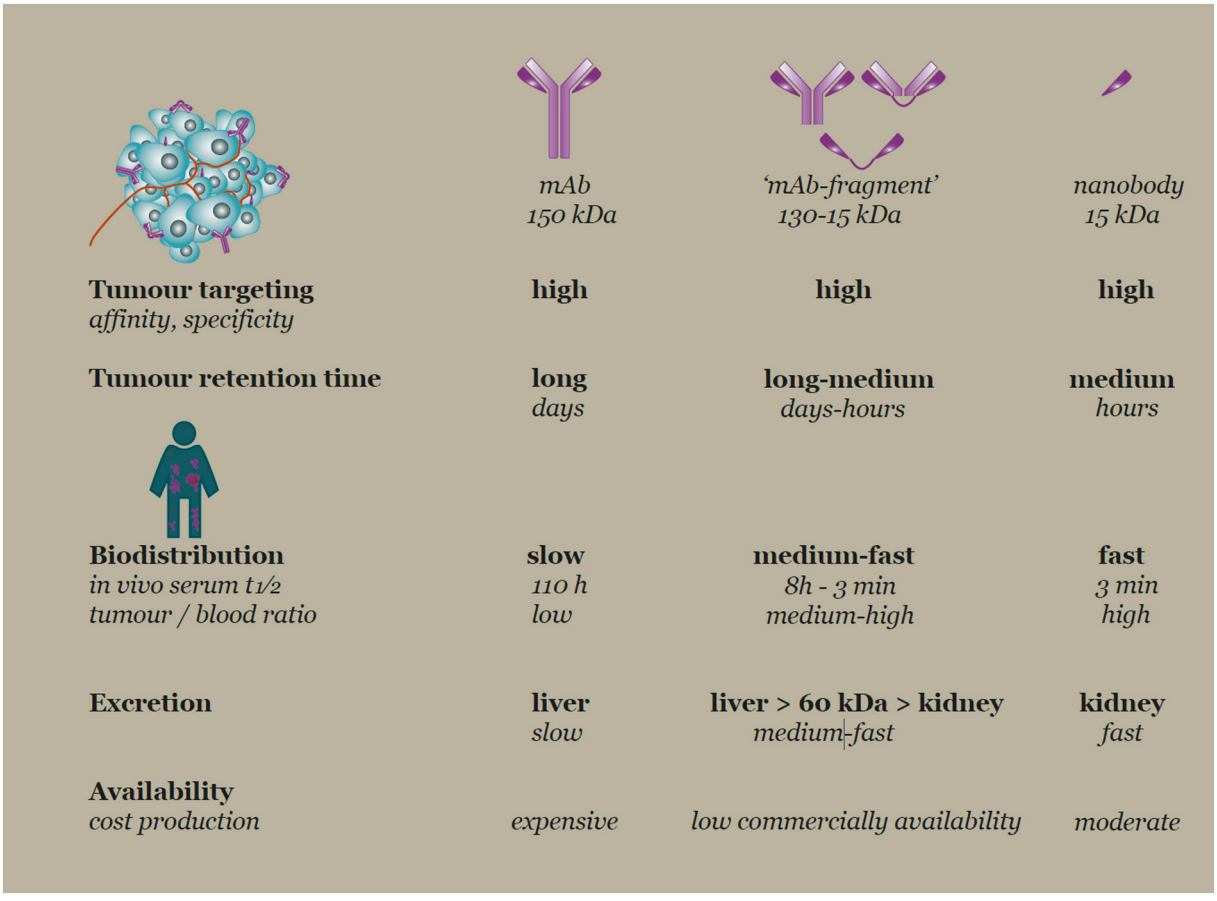


**Fig. 1.** Schematic representation and characteristics of mAbs compared to mAb-fragments, pointing out nanobodies. The characteristics are respectively compared between mAbs and mAb-fragments.

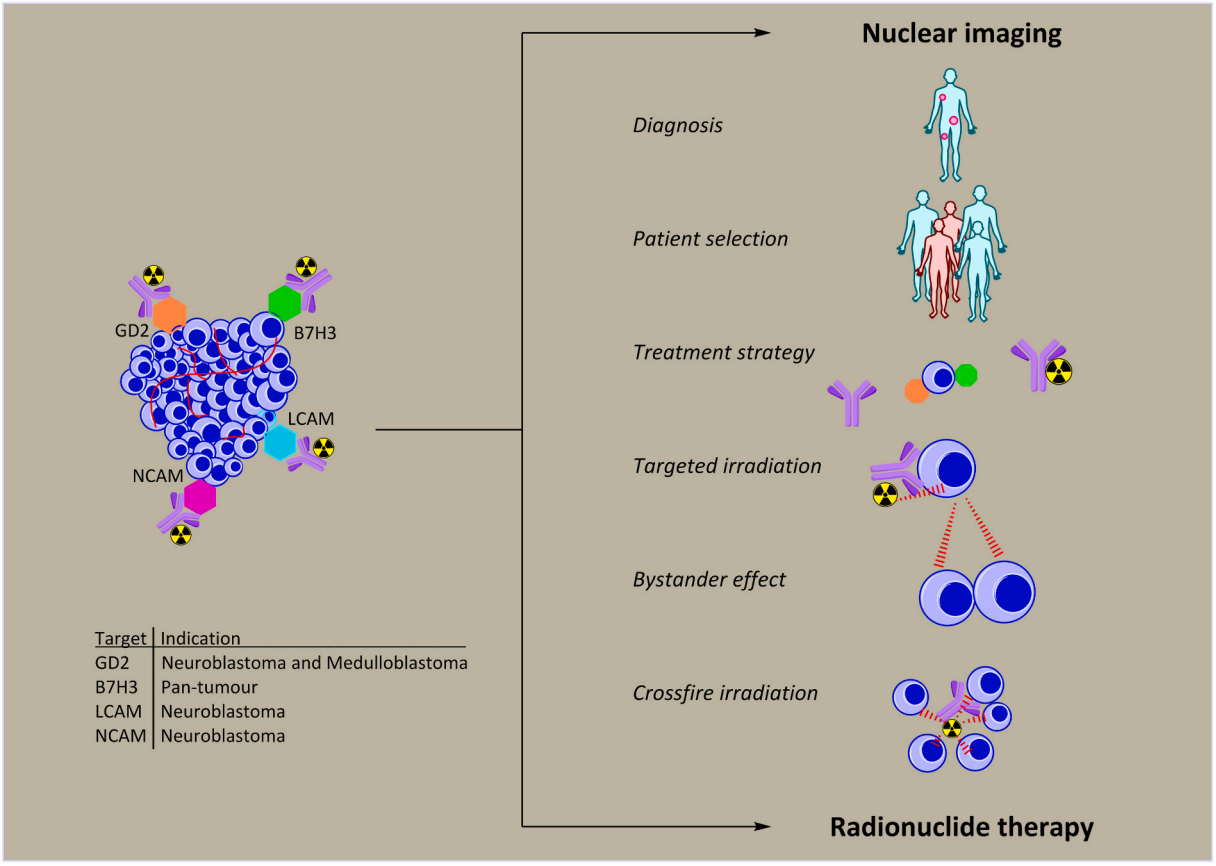


**Fig. 2.** Schematic representation of the application of diagnostic and therapeutic radiotracers. Diagnostic tracers in nuclear imaging can be used as diagnostic tool, for patient selection and therapy strategy. Therapeutic tracers in radionuclide therapy can kill the tumour by targeted irradiation, which causes DNA-damage, the bystander effect, and crossfire irradiation. Overall, a personalized medicine strategy can be achieved by nuclear medicine.

individual patients could benefit most from these mAb-mediated therapy [16]. Also, nuclear imaging is a non-invasive diagnostic tool, unlike often used immunohistochemistry (IHC) which requires patient biopsies. Therefore, developing radionuclide tracers that target immune checkpoint inhibitors has great potential. However, these tracers must meet clear requirements as discussed by Wierstra and colleagues [17]. The tracers must have high target affinity and low uptake in target negative tissue resulting in a clear 'on-target-off-tumour' distribution. Furthermore, the tracer must show stable *in vivo* behaviour. Developing radionuclide tracers that target immune checkpoint inhibitors comes with even more challenges, such as preserving normal immune function [17]. A personalized medicine strategy is achieved by nuclear imaging in combination with radionuclide therapy, to irradiate the tumour locally, causing tumour death with limited side effects for the surrounding tissue. In radionuclide therapy, the tumour becomes apoptotic due to three mechanisms: 1) targeted irradiation of the target cell, which causes DNA damage, 2) the bystander effect: surrounding cells are affected by signals such as death ligands, reactive oxygen species and cytokines from the irradiated cells, and 3) crossfire irradiation: surrounding cells are affected by radiation of the targeted cells, allowing heterogenous targeting [8]. Tumour specific targets with low expression on healthy tissue are needed for suitable radiopharmaceuticals. These targets can be expressed on the cancer cell itself but also in the tumour-microenvironment.

When developing a radiopharmaceutical, one should select the best possible vehicle for targeting which can be low molecular weight molecules, peptides or biologicals. The radionuclide that suits the desired purpose may be a positron or γ-emitter for diagnostic purposes and a β- or α-emitter for therapeutic purposes. The best possible chelator or prosthetic group strategy should be selected, depending on its application and radionuclide [18]. Knowing the application of the radiopharmaceutical is important for the selection of the type of vehicle. For example, an antibody fits well for diagnostic purposes, since it has a long retention time of days and high target selectivity. For radiolabelling with therapeutic nuclides and treatment, the circulation of intact mAbs is often considered too long, potentially causing off-target effects and long-term toxicity. Therefore, mAb-fragments such as nanobodies [19], affibodies [20], DARPins or adnectins [21] are considered more favourable because of their rapid circulation and fast target binding, maintaining the desired selectivity. Preferably the targeted cell-surface receptor internalizes upon binding of the radiopharmaceutical, potentially resulting in residualization of the radionuclide of choice. This results in an increased accumulation which can be beneficial to the signal-to-noise ratio for diagnostic nuclides or the potential treatment effect of therapeutic nuclides. Typically, radiometals are nuclides that will residualize in the cell [22].

The development of mAb-fragments is well described, and production costs are relatively low. Also, the products are considered less toxic and have a low immunogenicity, which could be further maximized by humanization [23]. Another important consideration for choosing between a mAb or a mAb-fragment is the 60 kDa renal cut-off. Fragments lower than 60 kDa molecular weight have a fast clearance by the kidney. Besides selecting the best vehicle, another consideration is selecting the best radionuclide. These vary in their radiation type (*i.e.* α, β, γ), half-life ($t_{1/2}$) and labelling strategy. For example, low molecular weight molecules are often labelled with the positron emission tomography (PET) nuclides fluorine-18 ($^{18}$F) and gallium-68 ($^{68}$Ga) and the single-photon emission computed tomography (SPECT) nuclide iodine-123 ($^{123}$I). Whereas for mAbs copper-64 ($^{64}$Cu), zirconium-89 ($^{89}$Zr) or indium-111 ($^{111}$In) are more suited because of their longer $t_{1/2}$. For therapeutic

applications, the β-emitters iodine-131 ($^{131}$I) and lutetium-177 ($^{177}$Lu) are often used. Increasing interest is shown in the α-emitters lead-212 ($^{212}$Pb), astatine-211 ($^{211}$At) and actinium-225 ($^{225}$Ac) because of their high Linear Energy Transfer, which is a short range in tissue in combination with a high energy deposition. This limits the toxicity to surrounding tissues and increases the toxicity for targeted tissue. Besides the radionuclide selection, the conjugation strategy is very important for the range of action and potency [18,24]. mAb(-fragments) can be conjugated 1) non-specifically at the lysine amino acids of the biological; resulting in a heterogeneous labelled product, or 2) site-specifically at the cysteine amino acids of the biological; resulting in a homogeneous labelled product. However, non-specific conjugation can result in too much conjugation of the biological that can obstruct the binding site. But site-specific conjugation is not always possible since cysteines are originally not present in mAb-fragments. Therefore, a conjugation strategy needs to be chosen. In conclusion, nanobodies and other antibody-fragments are very well suited as vehicles for theranostics applications. Similar to immunotherapy, the theranostics field is also switching towards nanobodies [2,19].

In adult oncology, several mAb-fragment-based theranostics have been studied for cancer targets such as CD20, EGFR, prostate-specific membrane antigen (PSMA), and mismatch repair (MMR). But the most clinically studied is the anti-HER2 2Rs15d nanobody for breast cancer patients [25]. $^{68}$Ga-labelled 2Rs15d as diagnostic tracer was safe and showed high tumour-to-background signal in clinical trials [26]. Also, the therapeutic counterpart which was labelled with $^{131}$I showed high specificity and effect in a phase 1 clinical trial (NCT02683083). Interestingly, preclinical results of 2Rs15d labelled with α-particle $^{225}$Ac looked very promising, which should provide higher tumour targeting and less toxicity for the surrounding tissue [27].

Overall, research in adult oncology has demonstrated that biologicals are excellent vehicles for molecular imaging and therapy. Also, the effect of radioimmunotherapy in several cancer types is currently investigated in clinical trials [8].

## 3. Current status of biological tracers in childhood cancers

The nuclear medicine field in paediatric oncology is limited in comparison to adult oncology. Until now, nuclear medicine is clinically mainly used for neuroblastoma patients, using *meta*-[$^{123}$/$^{131}$I]iodobenzylguanidine ([$^{123}$/$^{131}$I]mIBG) with a focus on optimizing this treatment [28,29]. Currently, [$^{131}$I]mIBG is incorporated in upfront treatment of high-risk neuroblastoma for patients with refractory disease (VERITAS study of the European consortia for neuroblastoma SIOPEN). In addition to mIBG, other $^{131}$I treatments are also available in nuclear medicine, *e.g.* for the treatment of paediatric thyroid cancers. The first radiolabelled mAb tested in children was [$^{89}$Zr]Zr-bevacizumab. Bevacizumab is a mAb targeting VEGF and was initially developed for colorectal cancer. Studies showed its potential in DIPG patients as well and consequently, bevacizumab is currently in clinical trials as potential immunotherapy for DIPG patients [30]. Jansen and colleagues performed the first [$^{89}$Zr]Zr-bevacizumab PET imaging in children; showing tumour uptake in DIPG patients and its potential as diagnostic agent for the selection of patients for this immunotherapy [31].

Besides the strategy of testing existing tracers used in adult oncology, paediatric target finding necessitates the development of novel childhood cancer specific radiotracers. This is important, considering that paediatric malignancies are different from adult cancers. A recent review by Kattner and Strobel discussed the difference in tissue of origin (*i.e.* blastoma *vs* carcinoma), cancer genetics, distribution, and microenvironment between adults and children [32]. They noted that leukaemia is the most common childhood malignancy, whereas it is the 12th most occurring cancer type for adults, and *e.g.* neuroblastoma and other blastomas are specific for children. Also, they discussed the difficulty in paediatric target finding, since paediatric cancers are not predominantly caused by genetic mutations, thereby expressing fewer cancer specific targets and only ~50 % of the patients express a druggable target [33]. New paediatric specific cancer targets need to be identified. There are several ongoing research programs, including Individualised Therapy (iTHER), that stimulate the drug discovery process and personalized medicine in paediatric oncology [34]. Until now, only a few paediatric specific targets were described that are used for theranostics applications. However, validated targets are, among others, the disialoganglioside GD2 and the immune checkpoint inhibitor B7-H3 that are highly expressed in childhood cancer and mainly studied for mAb targeting [35]. GD2 is a specific marker in neuroblastoma tumours and anti-GD2 treatment is included in high-risk treatment [35]. GD2 is therefore a valid biomarker for neuroblastoma and other tumours [35]. B7H3 is an immune checkpoint inhibitor with a high expression in several paediatric tumours [35].

In normal tissue GD2 is expressed on Schwann cells and synaptic junctions. Dinutuximab, an anti-GD2 humanized mAb, is the only immunotherapy used in clinic as standard care for paediatric solid tumours, and it induces the complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) against neuroblastoma cells. It is applied to prevent relapse in high-risk neuroblastoma patients. A published abstract in 2018 discussed the development of [$^{89}$Zr]Zr-dinutuximab which was demonstrated to specifically bind GD2 in neuroblastoma mice models [36]. Also, $^{64}$Cu-labelled dinutuximab showed preliminary tumour uptake *in vivo* [37,38]. Interestingly, the application of dinutuximab as vehicle for molecular imaging was recently demonstrated by Wellens et al., who developed the fluorescent IRDye800 labelled dinutuximab, antiGD2IRDye800CW, which showed high potential as new FGS tracer [39]. Different anti-GD2 mAbs are currently being investigated, including 3F8 and hu14.18K332A, which were also radiolabelled [40]. In 2012, hu14.18K332A was labelled with copper-64 and showed preliminary specific binding to GD2 and tumour uptake in osteosarcoma patient derived xenografts [41,42]. The mAb, 3F8 developed by Cheung et al., was used as vehicle for nuclear imaging. Radiolabelled 3F8 showed excellent tumour targeting in neuroblastoma patients using [$^{131}$I]I-3F8 and therapeutic response in neuroblastoma cells and rat xenografts using [$^{225}$Ac]Ac-3F8 [43,44]. Overall, biological tracers targeting GD2 were shown to be interesting molecular imaging modalities, which can improve the clinical outcome of neuroblastoma patients. However, as far as we know, it is challenging to generate mAb-fragments against the GD2 epitope since it is a glycolipid and not a protein. This makes GD2 a less suited target for radionuclide therapy. However, [$^{89}$Zr]Zr-dinutuximab as a diagnostic tool for patient selection and investigating relapse can be a desirable application.

Another highly expressed target on paediatric tumours is B7-H3 (*i.e.* CD276), an immune checkpoint inhibitor and known as a general cancer target. In healthy tissue, immune checkpoint proteins are expressed to regulate the immune response and prevent auto-immune reactions, but when (over)expressed on tumour cells this response is evaded. Therefore, mAbs inhibiting this evasion will result in tumour recognition by the immune system and killing of the tumour. The anti-B7-H3 mAb omburtamab (*i.e.* 8H9) is being investigated as a theranostic agent for neuroblastoma. Currently, [$^{124/131}$I]-omburtamab is in phase 1 clinical trials for neuroblastoma patients with CNS metastasis (NCT00089245) [45,46] and follow-up phase 2 and 3 clinical trials are planned (NCT03275402). Besides neuroblastoma, radiolabelled omburtamab is a potential theranostics for brain and CNS paediatric cancer types, such as DIPG (recruiting phase 1 and 2 clinical trials NCT01502917, NCT04167618, NCT04743661) and Desmoplastic Small Round Cell Tumour (recruiting phase 2 clinical trial NCT04022213). Particularly for DIPG patients, it was suggested that the diagnostic tracer [$^{124}$I]I-omburtamab is safe and feasible using convection-enhanced delivery. Also, the therapeutic potential of $^{177}$Lu-labelled omburtamab will be investigated in a clinical trial for another paediatric brain cancer, namely medulloblastoma (NCT04167618). In conclusion, biological

tracers targeting B7-H3 show great potential because of their high expression among paediatric cancer types and their potential as theranostics.

Another promising paediatric target is neural cell adhesion molecule (NCAM). NCAM (*i.e.* CD56) is a cell surface protein normally expressed in the nervous system, on natural killer cells and in lower extent on endocrine tissue, muscle tissue, adipose and soft tissue [47,48]. However, NCAM is also highly expressed on several tumours, and plays a crucial role in cell division, migration and differentiation during development. Also, NCAM expression in many cancer types predicts a more aggressive behaviour, more metastasis and a poor prognosis [49]. Publications from the 1980s showed clear preclinical and clinical neuroblastoma tumour targeting of [$^{123}$I/$^{131}$I]I labelled anti-NCAM mAb UJ13A [50]. However, it did not improve the existing and clinically used theranostics [$^{123/131}$I]mIBG [51,52]. Subsequently, in 2006, Otto and colleagues labelled the anti-NCAM mAb ERIC1 with $^{131}$I and demonstrated high affinity for NCAM [53]. Also, they showed *in vivo* tumour targeting in neuroblastoma mice model. However, there was no internalization of NCAM into the cell after binding of mAb ERIC1. This would be beneficial for the tumour targeting capacity.

Finally, the last promising paediatric target for molecular imaging and therapy is the cell adhesion molecule L1-CAM or LCAM, which is highly expressed in neuroblastoma [54]. LCAM and NCAM are both highly expressed antigens in many paediatric tumours, with a low expression in normal tissues and are potential biomarkers and targets for paediatric imaging and treatment [54]. Similar to NCAM, LCAM is expressed in the nervous system and on adipose and soft tissue, but LCAM is also expressed on kidney, urine bladder and on sex organ tissues [47,48]. The level of expression on the tumour in comparison to healthy tissue needs to be taken into account to determine toxicities. Anti-LCAM mAb chCE7 showed *in vivo* therapeutic effect and preliminary imaging of neuroblastoma patients with [$^{131}$I]-chCE7 [55]. Also, mAb-fragments of chCE7 were labelled with $^{177}$Lu and $^{64/67}$Cu, showing diagnostic and therapeutic effect *in vivo* [56]. To date, there were no follow-up publications on the use of chCE7 therapy for neuroblastomas or for other paediatric cancers. More recently, endosialin (CD248) was published as tumour biomarker in neuroblastoma and sarcomas. mAb-fragments targeting endosialin, scFv78-FC, were therefore recently $^{111}$In-labelled and investigated in neuroblastoma preclinical models where target binding was demonstrated [57].

## 4. Discussion and future perspective

This review describes the potential of radiolabelled biologicals, both mAbs and mAb-fragments, for molecular imaging and therapy of paediatric oncology. The availability of diagnostic tracers alone will allow improved diagnosis, patient selection and treatment strategy. The combination with therapeutic nuclides will give rise to new opportunities to treat childhood cancer. Both nuclear imaging and therapy improve the clinical outcome for children with cancer, of whom across all childhood cancers at least 25 % will not survive their disease. The only in standard care used biological for therapy in paediatric solid cancers, dinutuximab, is effective for most neuroblastoma patients [58–60]. However, side-effects are severe with patients suffering from neuropathic pain and 33 % of the patients suffer from relapse followed by death [61]. Therefore, it is important to develop a diagnostic tracer (*e.g.* [$^{89}$Zr]Zr-dinutuximab) to determine which patients could benefit most from this immunotherapy.

There are clear opportunities in the development of biological tracers for paediatric oncology. First, theranostic research has shown its potential in adult oncology, but in paediatric oncology there has been a lack of development so far. Many available biologicals for validated targets can be radiolabelled and evaluated preclinically, since the only theranostic currently used is [$^{123/131}$I]mIBG for neuroblastoma patients. We believe that novel theranostics, also for other paediatric cancers, can be a valuable tool for diagnosis, biomarker validation and treatment, resulting in an improved clinical outcome for the patient.

There are still many challenges in developing radiopharmaceuticals for paediatric oncology. A general consideration when applying diagnostic radiopharmaceuticals is the radiation burden for the patients. This especially holds true for longer lived radionuclides that are often used to radiolabel biologicals. For $^{89}$Zr-labelled bevacizumab, Jansen et al. calculated the radiation burden for DIPG patients in an age range of 6 to 17 years old that were injected with a dose of 0.9 MBq/kg up to a maximum of 37 MBq. This resulted in an average radiation dose of 22 mSv, which is considerably high. For future clinical translation of $^{89}$Zr-labelled biologicals, and for other diagnostic radionuclides used in children, dosimetry and radiation burden need to be addressed to further evolve this field of research [31]. The new generations of PET scanners and especially the development of total-body PET scanners might be the solution for the high radiation burden to the patients. Initial study results of total-body PET scanners with $^{89}$Zr calculated that the injected dose can possibly be reduced a 10-fold, potentially diminishing the radiation burden to the patients [62]. Another challenge for the development of theranostics in this patient population is the low occurrence of paediatric cancer types making it difficult to evaluate paediatric specific targets, validate new drugs and provide a fast translation to the clinic. Also, the development of immunotherapy for children remains challenging, as described by Casey and Cheung [35]. These challenges include an immature immune system, the risk for toxicity due to an overacting immune response and lack of predictive biomarkers for immunotherapy. However, especially the last challenge can be overcome by using nuclear imaging to select the patients who will benefit from a certain treatment. Also, antibodies are difficult and expensive to produce, which makes it challenging to use as a nuclear imaging vehicles.

The development of therapeutic radiopharmaceuticals comes with even more challenges. Whereas low doses are needed of the diagnostic tracer for generating a PET or SPECT scan, the efficacy and safety of the therapeutic tracer needs to be investigated. Also, more research is needed for defining paediatric tumours as radiosensitive or radioresistant. This will be important in defining the treatment strategy. Interestingly, a publication from 2016 indicated that LCAM expression enhances radioresistance of *MYCN*-amplified neuroblastoma cells (*i.e.* high-risk neuroblastoma) [63]. And a recent study showed a correlation between *MYCN*-status and the effect on radiotherapy upon glutamine deprivation: *MYCN*-amplified cells were radioresistant, whereas *MYCN* non-amplified cells were radiosensitive [64]. The level of radiosensitivity or radioresistance is important in defining the treatment strategy. Also, the radiation burden of the radiopharmaceuticals needs to be within limits. One should consider the potential long-term effects in children. Furthermore, a good consideration needs to be made for choosing a mAb or a mAb-fragment as vehicle. Overall, a better understanding of the radiobiological effect and the level of radiosensitivity or radioresistance is needed and additional studies are called for to investigate the safety and efficacy of biological tracers in children.

Despite these challenges, mAb-fragments have great potential as theranostic vehicles as they combine the fast circulation and targeting of low molecular weight molecules and peptides with the high affinity and specificity of mAbs. Reducing the molecular weight of biological tracers is not the only way to reduce the patient's radiation burden and optimize the radionuclide's pharmacokinetic profile. Pretargeted imaging and radioimmunotherapy (RIT) is an emerging field within theranostics research. In these methods first the tumour is targeted with an unlabelled antibody construct and second the tumour is targeted with a radiolabelled small molecule [65].This will result in a more rapid clearance of the radioactivity from healthy tissues. Pretargeting is a promising field, but optimization is needed with respect to tumour uptake of the radionuclide and excretion of the unreacted small molecule to translate this method into the clinic [66–68].

To date, the main biologicals that are used for nuclear imaging and therapy in paediatric oncology are anti-GD2 and anti-B7-H3 mAbs.

However, for a theranostic approach, the nuclear medicine field is switching towards the use of nanobodies and other mAb-fragments because of their favourable *in vivo* behaviour. Therefore, future work should concentrate on the diagnostic and therapeutic application of currently investigated biological tracers. To increase the potential of the radiolabelled anti-B7-H3 mAb omburtamab, a mAb-fragment might be preferred for the previously described reasons. For GD2, however, it is difficult to develop a mAb-fragment because of its glycolipid characteristics. Also, future work should investigate the development of new biological tracers for paediatric oncology. Another highly expressed tumour target in paediatric cancer types is NCAM. Anti-NCAM nanobodies are commercially available. They can be labelled and should be investigated as vehicles for molecular imaging and as therapeutic radiopharmaceuticals.

In the future, molecular imaging techniques will be integrated more frequently in clinical care. For example, in neuroblastoma patients, a diagnostic PET scan could be performed using [$^{89}$Zr]Zr-dinutuximab to determine the distribution of dinutuximab before the actual treatment. Next, a treatment strategy can be defined and FGS can be performed using antiGD2IRDye800CW to remove the tumour, which can be checked post-treatment by another PET scan.

In conclusion, the development of new imaging and therapy strategies based on biological tracers is essential to improve the clinical outcome for children with cancer.

## Funding

Sponsored by UMC Utrecht and Princess Máxima Center for Paediatric Oncology, Netherlands.

## CRediT authorship contribution statement

Conception and design (V.J.A.N., S.T.M.W., A.J.P.); writing - drafting article (S.T.M.W.); writing - review and editing, all authors (V.J.A.N., S.T.M.W., A.J.P., M.M.v.N., M.G.E.H.L.), visualization (S.T.M.W.); supervision (A.J.P., M.M.v.N., M.G.E.H.L.); All authors have read and agreed to the published version of the manuscript.

## Conflict of interest

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.

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