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The contribution of Nanoparticles in diagnosis and treatment in Pediatrics

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1 Abbreviations

AFt	Apoferritin	LOD	Level of Detection
AgNPs	Silver Nanoparticles	MB	Medulloblastoma
AIDS	Acquired Immunodeficiency Syndrome	miRNA	Micro RNA
AML	Acute Myeloid Leukemia	MRI	Magnetic Resonance Imaging
ARG	L-arginine	NHP	Non-human Primate
AUC	Area Under the Curve	NPs	Nanoparticles
AuNPs	Gold Nanoparticles	PACC	Pediatric Adrenocortical adenoma
BBB	Blood Brain Barrier	PBAEs	Poly(beta-amino ester)s
CNDs	Carbon Nitrate dots	PBNPs	Prussian Blue NPs
CNS	Central Nervous System	PCR	Polymerase Chain Reaction
CSCs	Cancer Stem Cells	PET	Positron Emission Tomography
DHEAS	Dehydroepiandrosterone Sulfate	PTPmu	Protein Tyrosine Phosphatase mu
DIPG	Diffuse Intrinsic Pontine Glioma	PTT	Photothermal Therapy
DSA	Digital Subtraction Angiography	PZQ	Praziquantel
EPR	Enhanced Permeability and Retention Effect	RES	Reticuloendothelial System
EVs	Extracellular Vehicles	RSV	Respiratory Syncytial Virus
FDA	Food and Drug Administration	siRNA	Small interference RNA
FNH	Focal Nodular Hyperplasia	SPARC	Secreted Protein Acidic -Cysteine
GAS	Streptococcus Pyogenes	STING	Stimulator of the Interferon Genes
GBM	Glioblastoma	T2WI	T2 Weighted Images
GM	Gemcitabine	TB	Tuberculosis
HIV	Human Immunodeficiency Virus	TMZ	Temozolomide
HSVtK	Herpes Simplex Virus Type1 thymidine Kinase	Tf	Transferrin protein
LAM	Glycolipid Lipoarabinomannan	VOCs	Volatile Organic Compounds
LAMP	loop-Mediated Isothermal Amplification	ZIFs	Zeolitic Imidazole Frameworks
lncRNA	long non-coding RNA	18F-FDG	18F-fluorodeoxyglocose

2 Abstract

Nanomedicine has evolved greatly the last decades and expanded its applications in Pediatrics. A lot of research has been applied on both the diagnostic procedures and treatment. This review presents the newest research focusing on their impact in the everyday practice of the clinician and the quality of life of the young patient. Pediatrics represents a special domain with unique patients and different needs. Children differ not only regarding pharmacokinetics and pharmacodynamics but they also develop at the time of the treatment. Meanwhile, their compliance and cooperation are not always guaranteed and a variety of techniques have to be used to achieve that. The sample collection and imaging procedures are of significant difficulty. Nanoparticles come as a beacon of hope to provide sensitive and specific diagnostic tools, novel contrast agents like ferumoxytol could potentially eliminate the need for sedation during scans. They also provide new tools to minimize the radiation the oncology patients receive thru their sensitizing abilities. They act as carriers for nucleic acids against aggressive types of cancers and antibiotics against the even rising antibiotic resistance. In addition, children require special forms of medication such as syrups and powder forms, which are not currently available for a lot of important medications. Nanoformulations can give an easy and inexpensive solution to this problem. The limitation that research faces involve mainly the lack of pediatric models and the skepticism around nanoparticles. While the attention has been mainly in oncology, other domains can benefit even more from the use of nanoparticles.

3 Introduction

Nanoparticles have already conquered a lot of domains of conventional medicine. From vaccines to gene therapies and radiotherapy, almost every specialty has known the advantages of nanomedicine. Regarding pediatrics nanoformulations behold a great future ahead of them. Nanostructures emerge as a beacon of hope not only for the tretment of a great variety of until now untreatable diseases but also for the development of new diagnostic methods and also for the improvement of the already existing ones.

This review provides an overview to the current and most recent research around the applications of nanoparticles in pediatric diagnosis and treatment from the pediatrician's point of view. The clinical importance of each application is highlighted and the benefits at the day-to-day practice are mentioned. Additionally, the limitations that nanomedicine encounters in pediatrics are analyzed. Thru these two aspects, the current progress and obstacles that the researchers face, the future perspectives will also be presented.

Children represent a unique group of population especially when it comes to the administration of medications. Pharmacokinetics and pharmacodynamics differ greatly and every product must be adjusted to dosing adequate for them. In addition to that the form of the drug has to be formulated so as to be appropriate for children and also with a taste that will not only be nice but also attractive for them. This formulation has to be adjustable to dosing depending on the weight and age of the patient.

Syrups and sachets diluted in water are already used widely but only for a small number of drugs. Therefore, there is a huge gap in pediatric pharmacy regarding oral medication that can easily be filled by nanoformulations. Nanocarriers have successfully been used in order to create powder forms of drugs for water, juice or even milk dissolution[1]. Moreover, by utilizing a variety of nanotechnology techniques conventional drugs can be transformed into powders with the addition of a sweet component for a better flavor[2].

Pediatric oncology once again has been profited a lot. New types of therapies such as gene therapies, immunotherapies are developed constantly[3,4]. In addition to that some of the already used anticancer drugs, which until now had important side effects when administrated to children can be formulated in order to target the specific pathologic cell population[5]. The use of nanocarriers has facilitated this advancement by achieving a more targeted approach[5]. Controlled release is another advantage nanostructures can provide[4]. Both these properties minimize the toxicity[5].

Diagnostics have in general benefited greatly. Regarding microbiology rapid test have been evolved so as to give answers for a lot conditions fast and without expensive equipment[6]. Especially in infectious diseases they can minimize the cost and the time needed for a diagnosis to be made[7]. Sample collection is harder when it comes to younger patients, therefore an increased accuracy is imposed for pediatrics. A great number of biosensors are currently under investigation and the technology behind them is based in nanoparticles[6].

Radiology and imaging in general are traditionally a difficult aspect of pediatrics. Firstly, due to the lack of cooperation of the young patients, which leads to the use of sedation in order to receive images with good quality. Both the medications used for the sedation and the contrast agents cause either immediate side effects such as allergy reactionw or long-term ones especially regarding the patients which require multiple scans[8,9]. Ferumoxytol an iron oxide nanoparticle, already FDA approved can provide a long circulation time with no toxicity[8]. Its use as contrast agent has application in approximately every system, from metastases detection to pathologies of the cardiovascular system[8–10]. Moreover, the radiation received is often an important obstacle for the clinician.

Additionally, the impermeability of the blood brain barrier is a significant obstacle especially regarding pediatric oncology, where this type of tumors constitutes a great percentage of the cases. Nanoparticles either due to their small size or due to their hydrophobic structure can carry valuable drugs inside the brain. Ligands on their surfaces can target specific tumor cells and achieve therapeutic

effect with minimum toxicity on the developing brain[5]. Surface receptors like the transferrin one can facilitate the entrance of the nanoparticle inside the brain[11].

Children do not only differ regarding the dosage in medications but also at the side effects from the radiation they receive. The long tern of which are sometimes devastating. So, the need for minimum radiation is profound. Nanoparticles have been used successfully as either sensitizers for either radiotherapy or photothermal therapy[12–14].

Theranostics represent a new kind of procedure that combines both the diagnosis and therapy. Nanoparticles are the cornerstone for theranostics, as they can reach the pathologic tissue thru targeting and depict it by using an imaging technique and their imaging properties. At the same time, they are able to release the drug or active substance, they carry, to the desired location[15]. Pediatrics require the less innervations possible. Children do not cooperate; they need time and special conditions to feel comfortable. Once their cooperation is achieved it is better to perform both the diagnostic test and the therapeutic intervention.

Vaccines have already integrated the nanoparticles either as carriers of the antigen or as adjuvants. Lipid nanoparticles were the ones used in covid 19 vaccine proved their safety and efficacy as carriers of the nucleic acid and also as immunostimulants for a better response and antibody production. After the covid 19 pandemic nanoparticles have been established as components of vaccines and a lot of research has been conducted regarding them. Therefore, such applications will not be addressed and the focus will be shifted to domains and applications that are currently investigated.

4 Theory

4.1 Nanoparticle Classification

Nanotechnology has been evolved greatly the last decades. On this ground nanomedicine has also gained significant progress. Nanoparticles have a size between 1 to 100nm, which provides them with particular properties. Depending on their composition nanoparticles are divided into three categories; organic, inorganic and carbon based[16]. Each with different characteristics and applications[16].

Organic nanoparticles, such as liposomes and dendrimers, are more biocompatible and also biodegradable[16]. At the same time, they have a lot of applications as drug carriers and vaccine components. The are characterized by an important sensitivity in heat and radiation, enabling the controlled release of the drug[16].

Carbon based nanoparticles like carbon quantum dots have extremely low toxicity[16]. They are mainly used in imaging and as drug carriers[16]. Their electrical conductivity combined with their special properties regarding light and heat absorption enables all these applications[16].

Inorganic nanoparticles include the rest of the nanoparticles[16]. A very wide and promising category are the metal nanoparticles. Their electrical and magnetic properties make them perfect candidates for imaging application and use as contrast agents[16].

4.2 Nanoparticles as components of sensors

Sensors are devices which detects molecules in variety of samples, including blood and pharyngeal swab. The amount of sample needed is usually small and the result is available in a few minutes. A very important feature for pediatrics, where the collection is usually harder and the amount always less than those of the adults. In addition to that, the test can be conducted near the patient and no special equipment is needed, which lowers the cost significantly.

Nanoparticles due to their special properties are perfect candidates for the design of sensors. Their surface can be formulated and linked with antibodies or other molecules in order to bind with the desired substance. Gold nanoparticles have already been used for such applications and are currently investigated for more[6].

4.3 Ferumoxytol

Ferumoxytol is an FDA approved medication, used mainly to treat iron deficiency anemia in patients with chronic kidney disease[8,17]. It is an iron oxide nanoparticle with hydrodynamic diameter of 17–31 nm. While the core consists of iron oxide the coating is made from polyglucose sorbitol carboxymethyl ether[17]. It is biodegradable, biocompatible and has a long intravascular half-life time of approximately 14 hours[8].

Additionally, it exhibits the ability to accumulate at the reticuloendothelial system[8,17,18]. Due to its unique characteristics, it has been used of label as contrast agent in MRI with a great variety of diagnostic applications and advantages in comparison with the conventional gadolinium-based contrast agents[17–19]. The gadolinium-based ones have two significant disadvantages. Firstly, they have been found to form depositions in the brain and secondly, they could cause significant damage to the kidneys of patients with chronic kidney disease[20].

The long-time effects of these depositions have not been investigated yet, nevertheless oncology patients and especially children require multiple scans[21]. Additionally, the developing brain of the young patients is more vulnerable to damage from such deposition. So, it is of great importance to develop new sensitive contrast agents with minimum side effects.

The long intravascular circulation time of ferumoxytol, especially in pediatric population gives the opportunity for breaks and extra time to calm the patients down and help them cooperate during the scan[8]. In this way the need for anesthesia is reduced, leading to not only lower cost of the examination but also to less side effects from the sedative medication. The feed and sleep method can also be applied. During which the contrast agent is injected and then the child is being

put to sleep[9]. Even if the procedure is disrupted there is enough time to continue without the need for a new injection[9].

Regarding its safety it requires an intact iron metabolization in order to be administrated. Although small doses of 5mg/kg do not lead to iron accumulation, its main contraindication includes patients with hemosiderosis and hemochromatosis, where the overload pre-exists[21]. At the same time, it is safe for use for patients with chronic kidney disease and does not cross the BBB[21]. Its polyglucose sorbitol carboxymethyl ether coating also creates some concerns regarding its use in patients with diabetes.

5 Methods

For the purpose of the research one platform was mainly used, Pub med. As key words the following were applied nanoparticles, nanomedicine children, oncology, therapy, diagnosis, infectious diseases, imaging. For the initial research a ten-year-old limit was applied and regarding the type of the articles both reviews and original papers were included. More than one hundred articles were collected. Therefore, a five years limit was introduced to exclude some of them. At the end forty articles constituted the initial bibliography, which was during the prosses enriched.

6 Results

6.1 Diagnosis

6.1.1 Imaging

6.1.1.1 Imaging in Brain tumors

The second most common type of pediatric cancer is the central nervous system (CNS) ones and specifically the brain tumors. At the same time, they hold first place regarding the most common solid tumors in children[15]. They are usually diagnosed with brain MRI using gadolinium us contrast agent. Based upon the

results and the stage classification the treatment plan consists of surgical resection, chemotherapy and radiotherapy.

Both the diagnostic and the therapeutic procedures are limited by the presence of the blood brain barrier (BBB). The BBB forms a protective wall for the brain by prohibiting the entrance of the non-essential molecules. It consists of tight junctions between the endothelial cells, the basement membrane, astrocytes, mural and immune cells[22]. As a result, the BBB forms a size depend barrier. Additionally, positive charged lipophilic molecules can overcome the BBB, whereas the entrance of other essential molecules is facilitated be specific membrane carriers[23].

The Gadolinium enhanced MRI constitutes the gold standard for the diagnosis and staging of the brain tumors. Nevertheless, the low permeability of the BBB decreases the sensitivity of the MRI and in some cases fails to detect cancer in its early stages or to fully depict the tumor and its limits. Additionally, gadolinium depositions have been found inside the brain parenchyma of all ages of patients, who underwent multiple scans. Taking into consideration that children are still developing organisms a lot of concerns arouse regarding its effect to their neurocognitive function. In addition to that its inability to successfully characterize the margins of the tumor is of great importance for some type of fast-growing malignancies where the exact limits have to be determined for the optimum surgical resection[23].

Glioblastoma (GBM) represents the most common brain tumor and at the same time one of the deadliests[24]. The evolution regarding its treatment and diagnosis has failed to increase the survival rates of the patients[25]. The reasons behind that involve the obstacles that the BBB creates and the inability to fully resect the tumor. Additionally, GBM cells have a highly invasive tendency[23,24]. Most of the currently used treatments have significant side effects on other organs, which may not be of great importance in adults but can cause severe problems on the developing organs of a child[15]. As a result, the need for both sensitive diagnostic tools and targeted therapy is profound.

Recently, it was established that pediatric and adult gliomas have significant different genomic and biological complexities[26,27]. Tumor genesis triggered

by oncogenic mutations, novel signaling pathways and different chromatin regulations represent some of them[27]. Which highlighters the need for more investigations regarding pediatric population and at the same time enables the use of treatments, which were ineffective in adult populations.

Covarrubias et all. Proposed the use of nanoparticles to target the Receptor protein tyrosine phosphatase mu (PTPmu)[15]. PTPmu can be found both at the main tumor itself and at the distal location of it. Iron Oxide nanoparticles formed into nanoparticle chains were conjugated with SBK2 peptide, which is the one that bind to the PTP mu, where used for MRI imaging purpose[15]. Liposomes conjugated with SBK2 peptide and fluorescence were used for optical imaging and histology examination[15].

The experiment included both in vitro and in vivo sections[15]. Both of them with positive results. As the PTP mu conjugated NPs detected the tumor with its margins and also the individual GBM cells that had migrated millimeters away from it using T2- weighted MRI. During the experiment the team marked the need for pediatric GBM models. Thus, they developed one using athymic mice to place SJ-GBM2 cells orthotopically. This model exhibited the special characteristics of the pediatric glioblastoma with great success.

The successful targeting of both proximal and distal GBM cells using PTP mu- NPs opens the road to theranostic applications. In addition, the combination of liposomes with iron oxide nanoparticles could facilitate a targeted delivery and release of the drug, controlled by an external low-power radiofrequency field. In this way the medication could cross the BBB and reach the great majority of the malignant cells, leading to a precise and effective treatment. Other possible biomolecules are investigated in order to be used as targets for a more precise diagnosis and treatment of GBM not only in Pediatric population but also in adults too[15].

6.1.1.2 Imaging in metastases and malignant lymph node detection

For cancer staging and treatment plan the metastases and malignant lymph node detection is a critical step. In general lymph nodes larger than 1cm are characterized as malignant. This assertation when applied in pediatric population shows low sensitivity. The fact that lymph nodes reacting to an infection can also be larger than 1 cm and the fact that smaller than 1cm can potentially be malignant, are to blame for this low sensitivity[28]. Additionally, the ^{18}F-fluorodeoxyglocose (18F-FDG) enhanced positron emission tomography (PET) scan, a technique widely used for the detection of metastases and malignant lymph nodes, is also affected by the reaction of the lymph nodes to an infection[18,29].

The team of Anne Monika Muehe and Florian Siedek used ferumoxytol as contrast agent in RET/MRI in order to distinguish malignant and benign lymph nodes in children diagnosed with cancer[18]. Ferumoxitol is an FDA approved iron oxide nanoparticle mainly used to treat anemia[18].

During the trial all the candidates underwent an integrated 18F-FDG PET/MRI scan[18]. The patients received two scans six months apart. Based on the second one the lymph nodes that remained the same were characterized as benign and the ones that changed were characterized as malignant. A histology evaluation took place only on samples received from two patients, where the solid tumor and the neighboring lymph nodes were surgically resected[18].

At the end of the trial the team came to the conclusion that ferumoxytol enhanced PET/MRI has high sensitivity regarding the characterization of malignancy in lymph nodes between 5mm and 10mm[18]. The enhancing hilum was the criteria and more specific the absence of it was indicative for malignancy[18]. Feromuxytol was well tolerated by the patients without significant side effects[18].

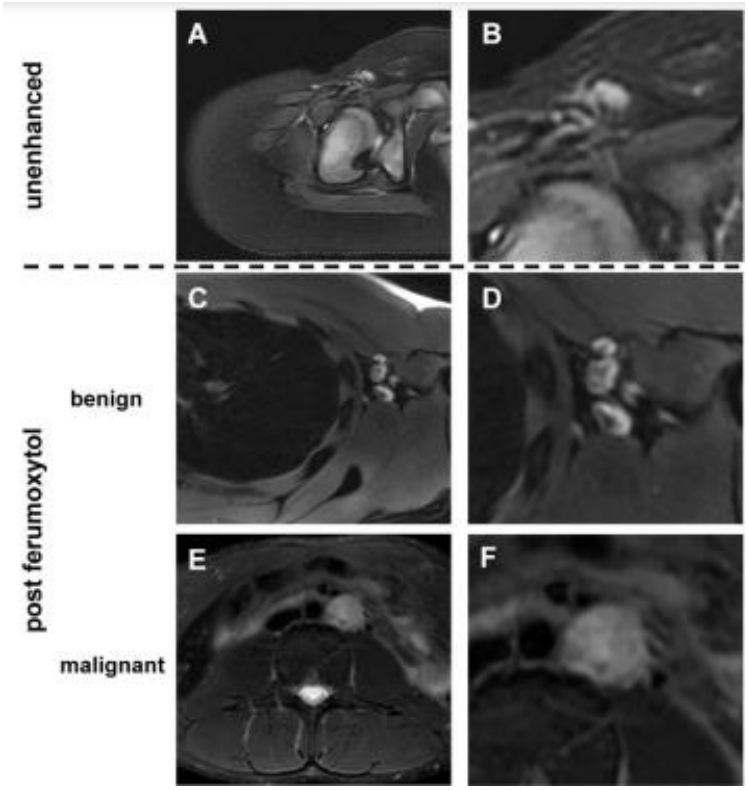

Figure 1 Morphology of benign and malignant lymph nodes on T2-weighted fast spin echo images. A and B represents the unenhanced images, C and D post Ferumoxytol images of benign lymph nodes, E and F post Ferumoxytol images of malignant ones Adapted from: Muehe AM, Siedek F, Theruvath AJ, et al. Differentiation of benign and malignant lymph nodes in pediatric patients on ferumoxytol-enhanced PET/MRI. Theranostics. 2020;10(8):3612-3621. Published 2020 Feb 18. doi:10.7150/thno.40606

6.1.1.3 Imaging in bones, soft tissue and liver tumors

The relatively large size of the NPs enhances the retention and permeability effect in cancerous tissues providing useful information for the differential diagnosis of benign and malignant tumors[30]. This ability is utilized for brain cancer imaging, with the additional advantage of assisting with the diagnosis of metastases[19]. On the other hand, the accumulation at the reticuloendothelial system (RES) allows an accurate depiction of tumors at liver, spleen and bone morrow, by producing a low signal at T2 weighted images (T2WI)[8].

Regarding bone and soft tissue cancer imaging ferumoxytol creates a more intense contrast between the healthy and cancerous tissue[8]. Additionally, it can detect bone marrow metastases due to the inability of them to take up the NPs, in contrary with the healthy tissue[21,31]. In comparison with gadolinium enhanced MRI the feromuxytol one provides higher quality and specificity[8].

The adult bone marrow presents with a fatty conversion leading to an easier detection of the metastases[17]. On the contrary the bone marrow of the children still possesses a great number of hematopoietic cells, which are really hardly distinguished from the metastases on MRI scans[17]. Nevertheless, it is still an organ of the reticuloendothelial tissue, which means that the leakage and the subsequent phagocytosis of feromuxytol from the macrophages also takes place there[17]. As a result, a negative signal in T2 weighted MRI is produced at the healthy sites, while at the focal metastases that does not occur[17].

On this ground Rashidi et all, compared the sensitivity of ferumoxytol enhanced and unenhanced MRI in the detection of bone marrow metastases in children and young adults[17]. All patients were between 2 and 25 years of age, diagnosed with extracranial solid tumors and one ore multiple bone marrow metastases with either the standard diagnostic procedures or biopsy. The scans were also repeated after chemotherapy was applied[17].

The results prove the increased sensitivity of the enhanced MRI (able to detect 99% of the metastases) against the unenhanced (able to detect 94% only of them)[17]. An interesting finding was the higher sensitivity of the post chemotherapy enhanced MRI, due to the increased hematopoietic activity of the bone marrow. The accurate detection of the metastases leads to a better staging and treatment plan. As a result, the survival rates also improve drastically[17].

Liver tumors and especially hepatoblastoma, the commonest one, are well depicted by ferumoxytol enhanced MRI, leading to detailed description of the tumor, its margins and its vascularization and providing a well-established treatment plan[32]. Another benign this time liver tumor, where ferumoxytol can assist with the diagnosis, is the focal nodular hyperplasia (FNH). In this case, the contrast agent results in the absence of the signal in T2 and T2* weighted images. This significant difference provides with an important diagnostic tool to distinguish it from the malignant lesions[33].

6.1.1.4 Imaging for sarcomas staging and treatment plan

The treatment plan for the sarcomas relies not only on the margins of the tumors but also on the neurovascular involvement and tumor thrombi[21]. Siedek et all, compared the sensitivity of gadolinium and ferumoxytol enhanced MRI regarding the depiction of bone and soft tissue sarcomas in pediatric population[21]. The results proved the equal ability of the two contrast agents to assess the margins and the size of the tumor[21]. Nevertheless, ferumoxytol provided more accurate information for the neurovascular involvement and tumor thrombi, which as mentioned above are crucial for the treatment plan[21]. Proving that Ferumoxytol can be used as gadolinium substitute adequately[21].

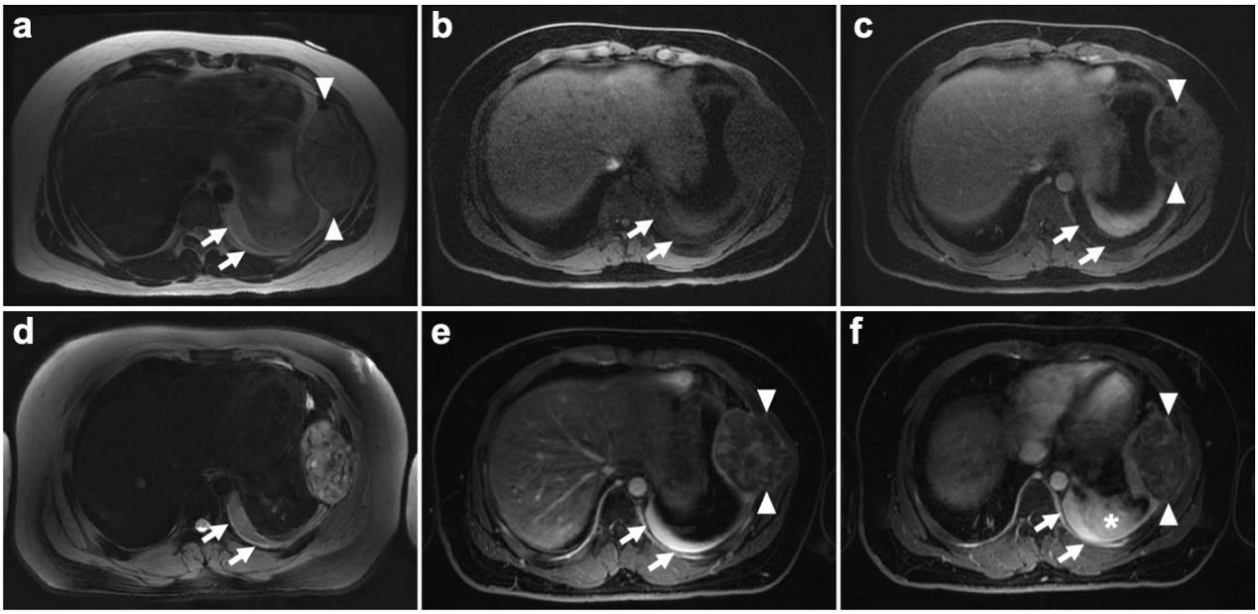

Figure 1 MRI scans of a patient 16 years old with a Ewing sarcoma in the 7th left rid (arrows). A T2 sequence, B and C before and after gadolinium administration, d 24 hours after Ferumoxytol administration, e and f LAVA sequences enhanced by ferumoxytol. Adapted from: Theruvath AJ, Rashidi A, Nyalakonda RR, et al. Ferumoxytol magnetic resonance imaging detects joint and pleural infiltration of bone sarcomas in pediatric and young adult patients. Pediatr Radiol. 2021;51(13):2521-2529. doi:10.1007/s00247-021-05156-y

6.1.1.5 Imaging for the detection of joint and pleural infiltration

Theruvath et all conducted a pilot study regarding the depiction of joint and pleural infiltration of bone sarcomas in young patients, both in children and younger adults[34]. Their study was based on the fact that malignant tissues produce exudate due to the increased permeability of the microvascular system of the synovium[34]. As a result, not only, small particles accumulate in the fluid but also

larger ones just like those of ferumoxytol[3536]. On the contrary, the reactive fluid caused by malignant tissues neighboring with healthy joints does not contain NPs. The microvascular structure of the joints that the tumor does not infiltrate is maintained and does not allow the bigger nanoparticles to cross it.

The MRI scans carried out 1 hour after the infusion showed no difference in signal between the healthy and the cancerous tissue, while the ones taken 24 hours later led to a significant enhancement of both the joints and pleural effusions in T1 MRI images of patients with tumor infiltration[34]. At the same time no or minimal difference in enhancement was noted in patients without spreading disease and in those with benign tumors[34]. This phenomenon was explained by the longer time that the silver nanoparticles required to accumulate at the site. The longer t1/2 time enables the scans to be taken 24 hours post- infusion, providing the essential time space for the accumulation[37].

In conclusion, ferumoxytol enhanced MRI can be utilized as a tool for a detailed characterization of the tumor margins around joints and chest wall leading to a better cancer staging and treatment plan. Especially regarding joints is of a great importance, because of the different surgical resection that a spreading disease requires and its results to the daily life of a young patient[3839].

6.1.1.6 Imaging in the Cardiovascular system

Ferumoxytol as contrast agent provides a long blood pool time which is of great importance for vascular imaging. Therefore, it is used in Magnetic Resonance Angiography, Venography and 4D flow sequences[8]. More specifically for the imaging of vascular anomalies with great results regarding the resolution[4010]. Cerebral arteriovenous malformations are a great example, where after the initial diagnosis with digital subtraction angiography (DSA) the use of ferumoxytol enhanced MRI for surveillance provides images with high resolution without the radiation[10].

In addition, it is an ideal contrast agent for the depiction of the vascular anatomy of both the donor and the recipient before and after a kidney

transplant[41]. Combined with its safety for the impaired renal system, ferumoxytol is the ideal contrast agents for children with either chronic kidney disease or acute kidney injury which are about to undergo a transplant or at need for imaging. Cardiac imaging including coronary vessels, congenital anomalies, myocardium structures can be significantly improved especially by 4D flow sequences[42–44].

6.1.1.7 Imaging in molecular and cellular level

Ferumoxytol as contrast agent has shown some promising results regarding the depiction in molecular and cellular level[8]. More specifically, stem cell implant rejection was diagnosed by the hypointense signal on T2WI, caused by ferumoxytol-label macrophages accumulations on the graft[45]. Additionally, by either activating ferroptosis or macrophages it could be used as a theranostic tool against cancer cells[46]. Although, such applications have not had enough investigation yet, they pose a great future perspective for the use of ferumoxytol[8].

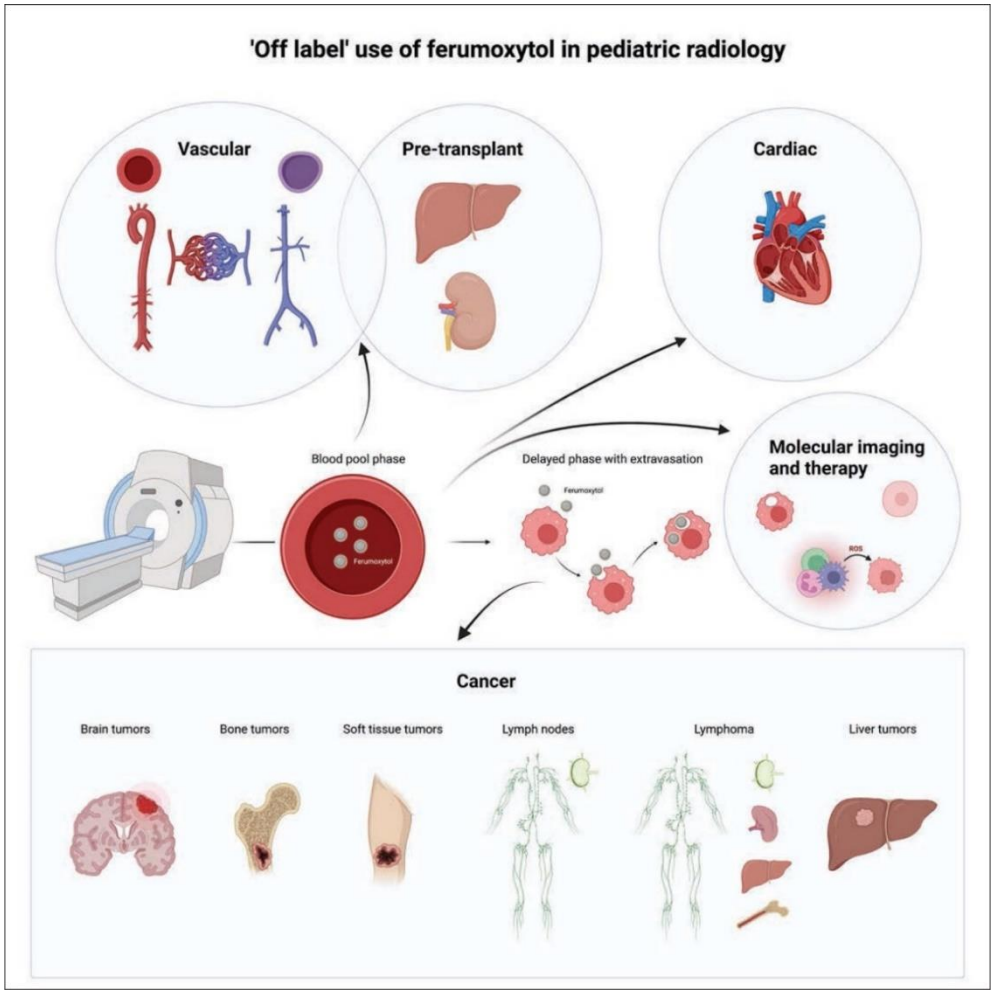

Figure 2 : Grafic illustration of the applications of ferumoxztol in pediatric Adapted from: Adams L, et al. Ferumoxytol-Enhanced MRI in Children and Young Adults: State of the Art. American Journal of Roentgenology (2023) 220(4) 590-603

6.1.2 Sensors – Point of care tests

6.1.2.1 Biosensor for the detection of Streptococcus pyogenes

One of the commonest complains among pediatric patients is the sore throat, especially combined with fever. Streptococcus pyogenes (GAS) a Gram-negative bacterium is a main cause of pharyngitis. Streptococcal pharyngitis is a self-limited disease, but when left untreated can lead to a severe autoimmune response called rhematic fever and also to post- streptococcal glomerulonephritis.

Its differential diagnosis from viral infections only by clinical criteria is usually hard, leading to an overprescription of antibiotics arousing concerns regarding antibiotic resistant. On the other hand, pharyngeal swab culture recures at least two days for a result which is an important delay for the treatment. Rapid tests

for the detection of GAS have already been developed, but their high cost combined with their instrument dependency limit their use[6].

Mohajeri et all, designed a biosensor utilizing gold nanoparticles for the rapid detection of GAS[6]. AuNPs were conjugated with H-1 antigens and aggregated as the result of the presence of GAS[6]. The commonest serotype of GAS causing pharyngitis is the M1. This characterization derives from the presence of the M1 protein a lectin that binds to the H-1 antigen[47]. The last one is a sugar code found in epithelial cells of the oral cavity[47]. Additionally, AuNPs with their tunable properties, easy synthesis and low cost provide ideal candidates for biosensor components[47].

Saliva sample was mixed with the conjugated AuNPs to observe the red shift in surface plasmon resonance position using UV- visible absorption spectroscopy[6]. Indeed, the samples containing M1 GAS caused a slightly noticeable at concentrations of 1 × 103 and 1 × 104 CFU/m, while at 1 × 105 and 1 × 106 CFU/ml the change was profound from red to purple color[6]. The whole procedure lasted only 20 minutes for each sample. At the same time, the sample that contained M12 GAS, M6 GAS and E.coli did not cause any alteration at the color[6].

This biosensor achieved a higher sensitivity in comparison with the already used rapid tests, which are able to detect GAS at concentrations between 9 × 103 CFU/ml and 1 × 105 CFU/ml[6]. The cost of its production is lower making it more accessible for frequent use. It also does depend on the instruments used and provides not only with qualitative information regarding the presence of GAS but also with quantitative ones[6]. More studies and modifications have to be done in order for the biosensor to be able to recognize other not so frequent serotypes of the GAS, but nevertheless it is very promising tool for the pediatricians.

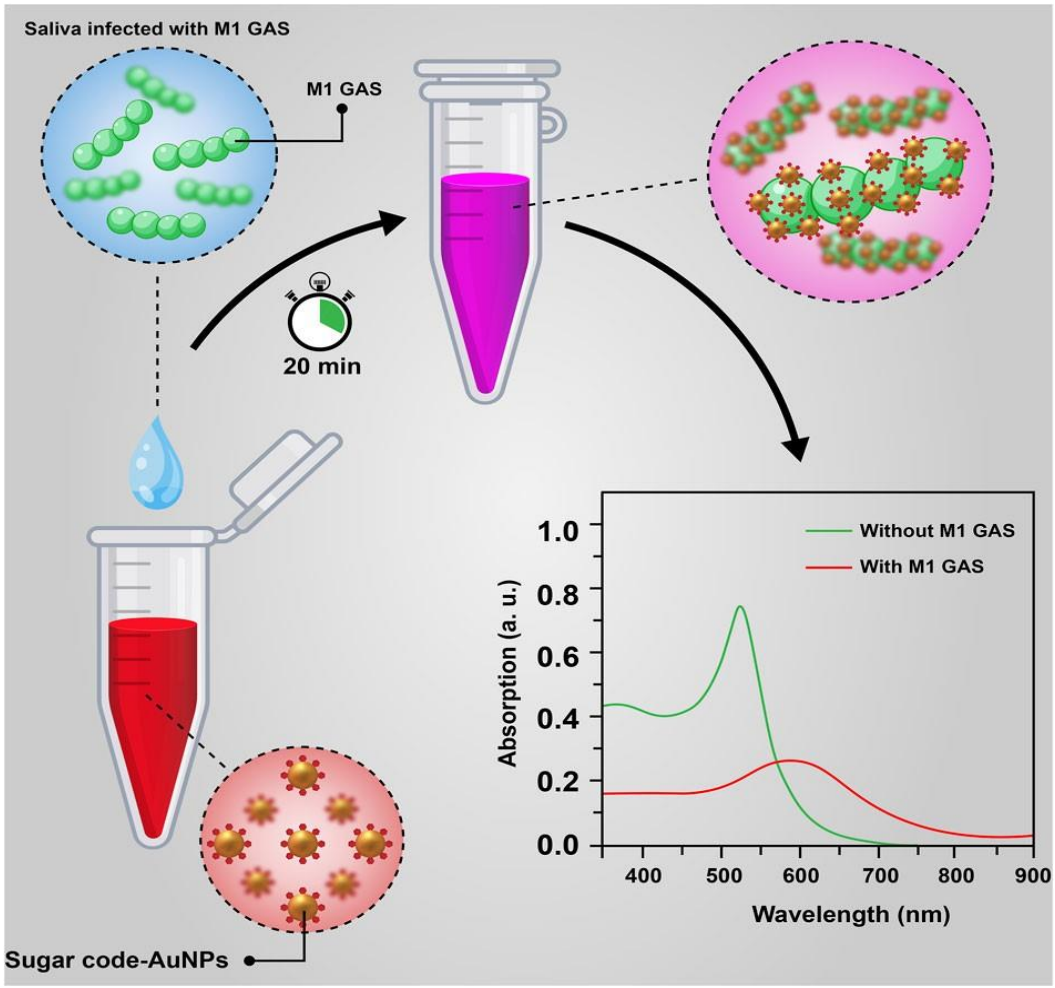

Figure 3 Schematic depiction of the procedure for the detection of GAS. The diagram presents the different absorption on a variety of wavelengths in samples with and without M1 GAS. Adapted from: Mohajeri S, Moayedi S, Azimi L, et al. Nanobiosensor Based on Sugar Code-AuNPs Aggregation: A Key to Opening New Gates in Rapid Diagnosis of Streptococcal Pharyngitis. Front Bioeng Biotechnol. 2022;10:957271. Published 2022 Jul 22. doi:10.3389/fbioe.2022.957271

6.1.2.2 Lateral flow biosensors for the detection of Bordetella Pertussis

Pertussis is a disease which mainly involves the upper and lower respiratory system. It most common cause is Bordetella pertussis. Although there is a vaccine against its short epidemics have been reported for time to time, due to incomplete vaccination of specific population groups. Pertussis has three stages the catarrhal, similar to common cold, the paroxysmal, which is the most dangerous and the convalescent. Pertussis can be fatal for neonates and small infants, which very often have not even had their first vaccine yet, as it usually administrated after the. For the detection of B. Pertussis PCR is the golden standard, which is really expensive and time consuming.

Sun et all, designed a later flow biosensor, with gold nanoparticles for the fast detection of B. Pertussis DNA after loop-mediated isothermal amplification (LAMP)[48]. The method was compared with PCR with better results, proving its better sensitivity. At the same time, it only requires one hour for the results, which is significantly less than the PCR[48]. Firstly, the DNA extraction takes place in 15 minutes, then the LAMP needs 40 min and at the end the biosensor in only two minutes provides the result[48]. Therefore, it can successfully be used as a diagnostic tool for pertussis.

6.1.2.3 Immunosensors for the diagnosis of adrenocortical adenoma in pediatrics

Pediatric Adrenocortical adenoma (pACC) is a rare but nevertheless aggressive type of childhood cancer[49]. The excessive secretion of dehydroepiandrosterone sulfate (DHEAS) is its main feature. The lack of symptoms at the first stages of the diseases complicates the diagnostic procedure[50]. In addition, the high aggressiveness of the later stages decreases the survival rate[50]. The early diagnosis would significantly improve the chances for survival, a key component to this is the early detection of DEHAS in plasma samples. Lima et all, engineered an immunosensor able to achieve this with great sensitivity and specificity[49].

Gold nanoparticles (AuNPs) due to their increased electrical conductivity and surface area provide ideal candidates for such applications.

Additionally, their preparation is significantly easier in comparison with other NPs, leading to an also easy functionalization, in this case with L-arginine (AuNPs-ARG)[51]. AuNPs-ARG connected anti-DHEA IgM antibodies with the oxidized glassy carbon electrode creating the immunosensor ox-GCE/AuNPs-ARG/IgM. AuNPs-ARG also amplified the signal received when DEHAS was connected with the IgM.

The clinical samples tested on the sensor were also tested with the electrochemical impedance spectroscopy with similar results leading to the conclusion that the immunosensor poses a great alternative to the detection of DEHAS with a Limit of detection (LOD) of 7.4 μg dL−1[49].

6.1.2.4 Tuberculosis (TB)

Tuberculosis (TB) is an infectious diseases caused by Mycobacterium tuberculosis (MtB). It affects mainly the lungs but also has other extrapulmonary manifestations, caused mainly by vascular spread like meningitis, peritonitis, pericarditis. TB is transmitted thru the air and inhalation, after the infection the patient can either develop active TB or latent TB. TB is one of the leading causes of death from infectious diseases in kids especially those under five years old[7].

TB diagnosis is currently based in the detection of the bacteria with either culture or Polymerase Chain Reaction (PCR) of sputum or the sample collected by the extrapulmonary location. Cultures take 2-6 weeks to provide the result[52]. On the other hand, PCR lacks sensitivity especially regarding Human immunodeficiency virus (HIV) positive patients[5354]. The situation gets even more complicated when the patient is a child. The samples often require more invasive procedures and get frequently disseminated due to lack of cooperation from the young patients[54]. Another obstacle regarding pediatric population is that the collected sample may contain only a small consecration of the bacteria, so a more sensitive method is required[52,53]. Additionally, distinguishing latent TB from TB disease remains a problem[55,56].

Zheng et al. used serum samples with nanoparticle enhanced immunoassays to detect extracellular vehicles (EVs) as biomarkers[52]. Glycolipid lipoarabinomannan (LAM) a key component of the cell wall and the membrane protein LprG were used as EV. Firstly, the LAM and LprG were evaluated as biomarkers[52]. Assay antibodies did not detect them in EV infected from other Mycobacterium or other pathogens, which led to the result that they are specific for Mycobacterium Tuberculosis. Secondly, their ability to distinguish latent Tb, from active Tb and from non-infected patients was tested. Non-human primate (NHP) models were used for this purpose, with very promising results. The signals of LAM and LprG were really low in health NHR, stronger in NHP with latent TB and really high in those with active disease[52].

The establishment of an inexpensive and fast diagnostic tool, which can distinguish latent and active TB, will contribute greatly to the reduction of the transmission of the disease[52]. An early diagnosis and intervention will also reduce the number of patients in need for hospitalization and as an immediate result the survival rates.

6.1.2.5 Nanoparticles as components of arrays for the diagnosis of acute leukemia in children

Leukemia is the commonest malignancy in pediatric oncology. First step for its diagnosis is blood sample evaluation also by microscopy. Bone marrow biopsy or aspiration confirms the diagnosis. A delayed diagnosis especially in children is attributed to its non-specific symptoms that can mimic common and usually non-life threating pathologies in young patients.

Volatile organic compounds (VOCs) represent carbon-based macromolecules produced by the cell metabolism and excreted to body fluids such as blood, breath and feces[57]. The type of VOCs produced vary greatly and depends on various factors. Cancer cells metabolism is forced to adjust to the increased needs of the rapidly multiplying rate of their cells[58]. As a result, the type of VOCs secreted differs from the healthy organism as shown at the table 1[58]. There are some excreted

only by healthy cells and some only by cancerous tissues while some of them are produced by both types at different concentrations.

Table 1: The different types of VOCs categorized based on the type of cell population, which produces them.

Healthy cells exclusively	Cancer cells exclusively	Both types of cells
methyl sulfide	hexanol	2,4dimethylheptane
4-methyldecane	cyclohexanol[58]	benzaldehyd
		o-xylene
		Hexanal
		Methylbeneze
		hexadecane
		3,7dimethyldodecane
		chloroform
		ethanol

VOCs are mainly detected and analyzed by gas chromatography an expensive and time-consuming method that also requires special equipment[59]. A sensor called E-nose was engineered to detect the VOCs easily and rapidly. Bordbar et all, utilized silver and gold NPs to create an array able to recognize VOCs from healthy individuals and patients with leukemia[57]. The sensor detected the blood vapor using 16 different NPs modified with eight different agents so as to be able to aggregate in the presence of a specific VOC and create a pattern for each sample[57].

The experiment conducted using blood sample form patients between 2 and 18 years of age diagnosed with leukemia with either aspiration or biopsy of the bone marrow and healthy participants also examined by a pediatric oncologist[57]. The only requirement for the patients were that they had not received neither chemotherapy nor radiotherapy. The presence of specific VOCs caused the aggregation of the NPs resulting to a color change on the assay and the creating of a

different pattern[57]. The ideal temperature and time required for the color change were also investigated (60°C for 4,5 hours)[57].

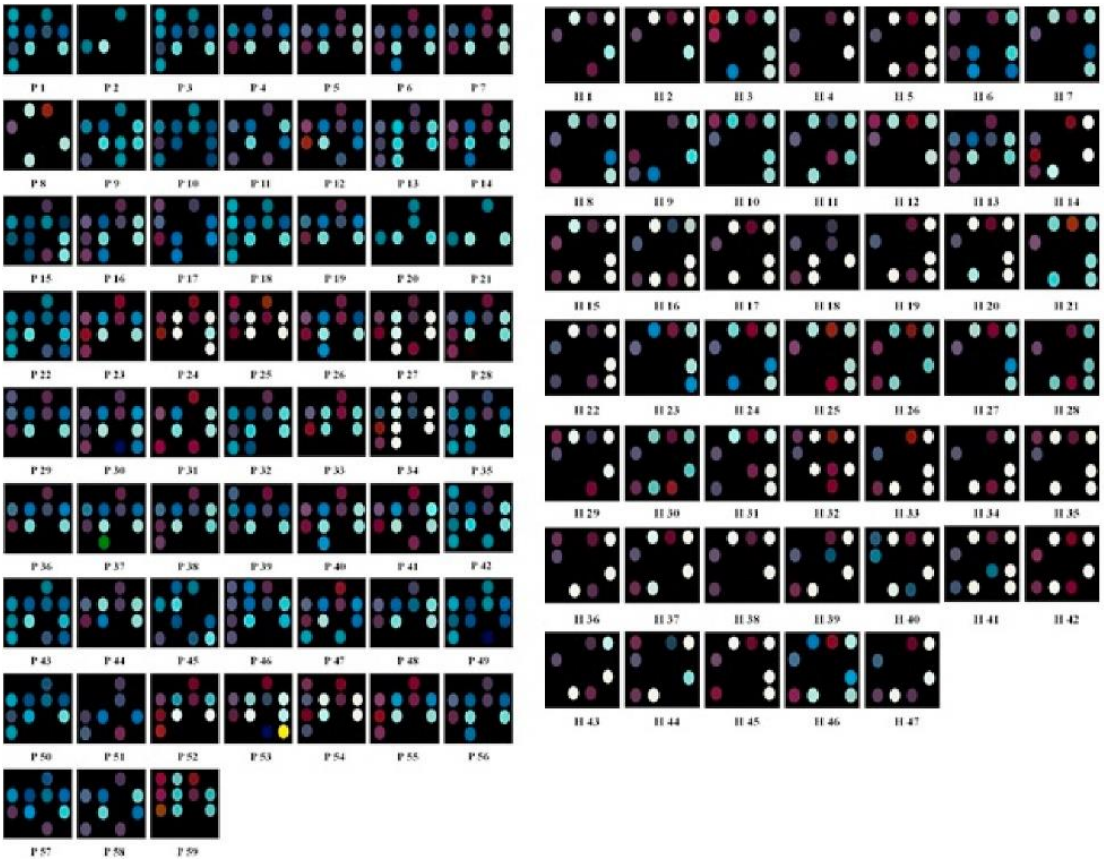

Figure 4 The patterns created from the interaction of the blood samples and the sensor. With H and P are the samples from healthy individuals and patients respectively. Adapted from: Bordbar MM, Barzegar H, Tashkhourian J, Bordbar M, Hemmateenejad B. A non-invasive tool for early detection of acute leukemia in children using a paper-based optoelectronic nose based on an array of metallic nanoparticles. Anal Chim Acta. 2021;1141:28-35. doi:10.1016/j.aca.2020.10.029

After the formation of the patterns, the arrays were used for principal component analysis by computer-based algorithms.[57] The patterns of the leukemia patients differed greatly from those of the healthy ones. The specificity, sensitivity and accuracy that arose from the experiment where really impressive, proving that the sensor could detect the patients, but not distinguish the different types of leukemia. More experiments have to be conducted in order to achieve that, but it is a first step for the formation of a fast, inexpensive and accurate screening for leukemia. Regarding it future perspectives it can be used as a tool for the evaluation of treatment response, but further investigation is required.

6.2 Applications of nanoparticles in pediatric treatment

Although pharmacy has made a huge progress during the last century, there is a huge gap regarding pediatric medications. The developing organism of the child leads to differences in both pharmacokinetics and pharmacodynamics. Therefore, drugs have to be evaluated again to define dosage and also to prove their safety. Although a lot of medication have been approved for children, there are not in formulations , where the dosage can easily be adjusted like syrups or powders. The administration is limited only in intravascular forms inside the hospital, creating a significant obstacle for the clinician.

In order to administrate some of them, when the dosage of the tablet form allows it, they are pulverized and then administrated with sugar water. This method requires a lot of effort, leading to decreased compliance. The bitter taste of the tablets is usually not covered by the sugar, decreases even more the amount of the medicine taken by the patient. To fill this gab in pharmacy nanoparticles and their technology are of great importance.

6.2.1 Treatment applications infectious diseases

6.2.1.1 Nanoparticle powder containing anti-HIV medication

Human Immunodeficiency Virus (HIV) is the main cause for acquired immunodeficiency syndrome (AIDS) a life-threatening condition. According to WHO 1.8 million children younger than 14 years of age were living with HIV at the end of 2019. The appropriate therapy regime includes 5 antiretroviral drugs. It should be started immediately after the diagnosis and continued for the rest of the patient's life in order to avoid the severe immunodeficiency that HIV can result into[60]. The compliance to the treatment plan is essential. However, it is hardly achieved in younger patients mainly due to the characteristics of the medication itself[1]. The bitter taste is usually an important obstacle for the pediatric population[1].

On this ground Dharshini et all, designed a chitosan nanoparticle as carrier for the anti-HIV drug dolutegravir that could be administrated inside the milk[1]. Dolutegravir acts by inhibiting the integration of the HIV DNA in the DNA of the

healthy cells[61]. Its main advantage is the long half-life time, which allows the once-a-day administration[1]. Nevertheless, its degradation inside the cells and tissues takes place fast, hence the need for a carrier to reassure that the medicine will reach its target intact[621].

Chitosan is a biocompatible and biodegradable material with no toxicity[63]. Additionally, it can be manufactured in great variety of sizes for the better transport of the selective drug[63]. In this case both synthetic and natural chitosan were used for the purpose of the experiment and in order to create solid forms the spray drying technique was used[1]. Firstly, the load capacity, the size, the stability, the z potential of the NPs was measured and later they in vitro part of the experiment took place in human T-lymphocytes cells cultures with the mixture of milk. The drug release, the antiviral effect and the toxicity were evaluated, with satisfying results[1].

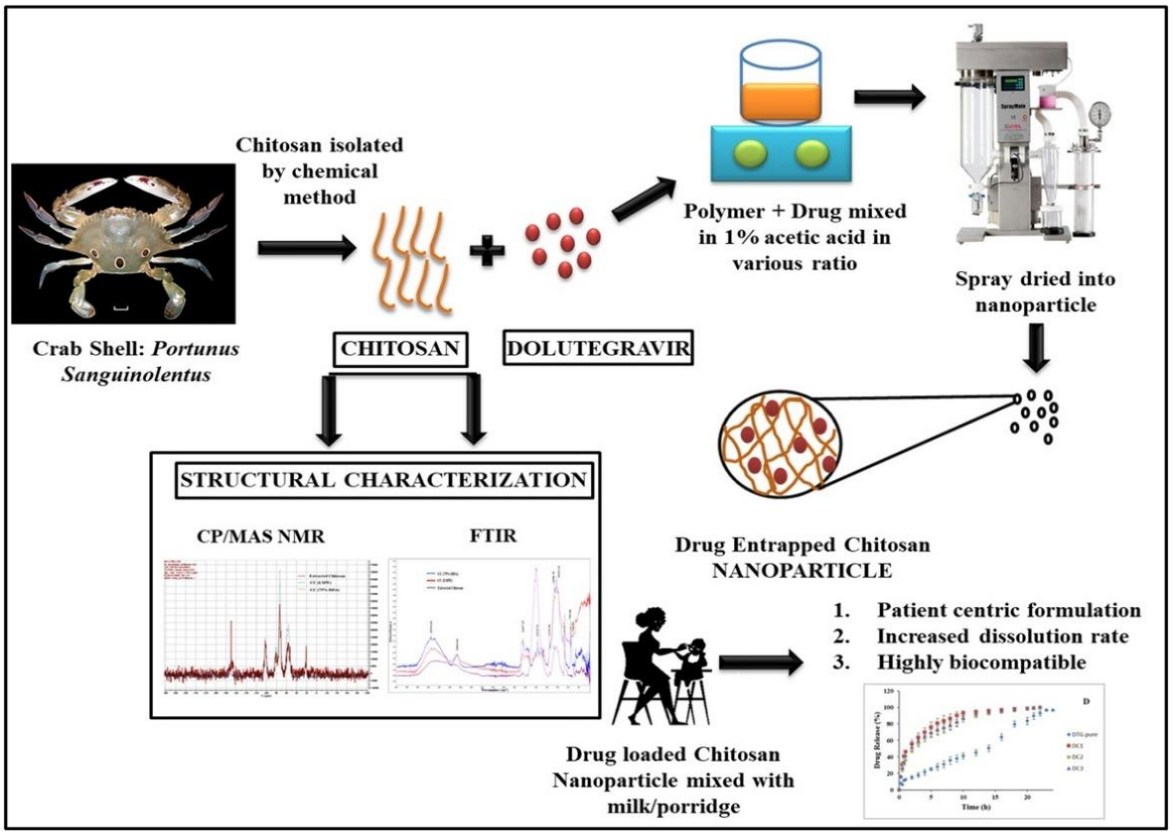

Figure 5 : Graphic depiction of the formulation of the chitosan powder containing dolutegravir. Adapted from: Priya Dharshini K, Fang H, Ramya Devi D, et al. pH-sensitive chitosan nanoparticles loaded with dolutegravir as milk and food admixture for paediatric anti-HIV therapy. Carbohydr Polym. 2021;256:117440. doi:10.1016/j.carbpol.2020.117440

For the in vivo testing Balb-C mice were utilized to set more light to the absorption, pharmacokinetics and biodistribution of the mixture of the formulation with milk and also compare them with that of the free drug[60]. The results indicated slower absorption of the NP-milk mixture, mainly due to the presence of the milk. Inside the gastrointestinal tube the secretions lead to the creating of complexes which require more digestion time and as an immediate result a slower absorption. Nevertheless, it did not affect the Cmax which was significantly higher than the one of the unprocessed drugs[60]. The same occurred with the area under the curve (AUC).

Regarding biodistribution the NP achieved a slower but a general distribution to the whole body including the brain, suggesting that chitosan can successfully cross the BBB[1]. A high concentration was also measured in uterus, an important factor for a sexual transmitted disease. The antiviral effect and low toxicity of the chitosan- drug formulation was once again proven, opening the road for the creating of more children friendly medication through nanoparticles to treat life threatening conditions[60].

6.2.1.2 Nanoparticles as tools against sepsis in pediatrics

Sepsis represents a dysfunction of the immune system caused by an infection. The activation of the sepsis mechanisms can have a catastrophic effect that often leads to death. Regarding its management in pediatric patients the antibiotic resistance as well as the underling and undiagnosed immune disorders combined with a delay onset of treatment create some obstacles that can be surpassed with the use of nanoparticles[64].

Lin et all, utilized zeolitic imidazole frameworks (ZIFs), which are biocompatible, stable with great porosity and surface area to act as carriers for the antibiotic metronidazole[65]. The most important characteristic of the ZIFs is their ability to release the incapsulated drug in response to low ph of the septic environment[65]. The nano dug named MI@ZIF-90 was firstly evaluated for its characteristics such as stability, physicochemical properties and loading capacity.

After receiving satisfying results its antibacterial, antifungal and anti-biofilm effect were evaluated in Escherichia coli, Staphylococcus aureus and Aspergillus fumigatus, Candida albicans cultures respectively[65]. Its ph response was the next one to be tested, with successful release of the drug in acidic conditions. Lastly its toxicity and hemolytic effect were assed to determine its safety[65]. The nano drug presented with great antimicrobial activity with no toxicity and minimum hemolytic reactions, proving the safety and efficacy of the formulation[65].

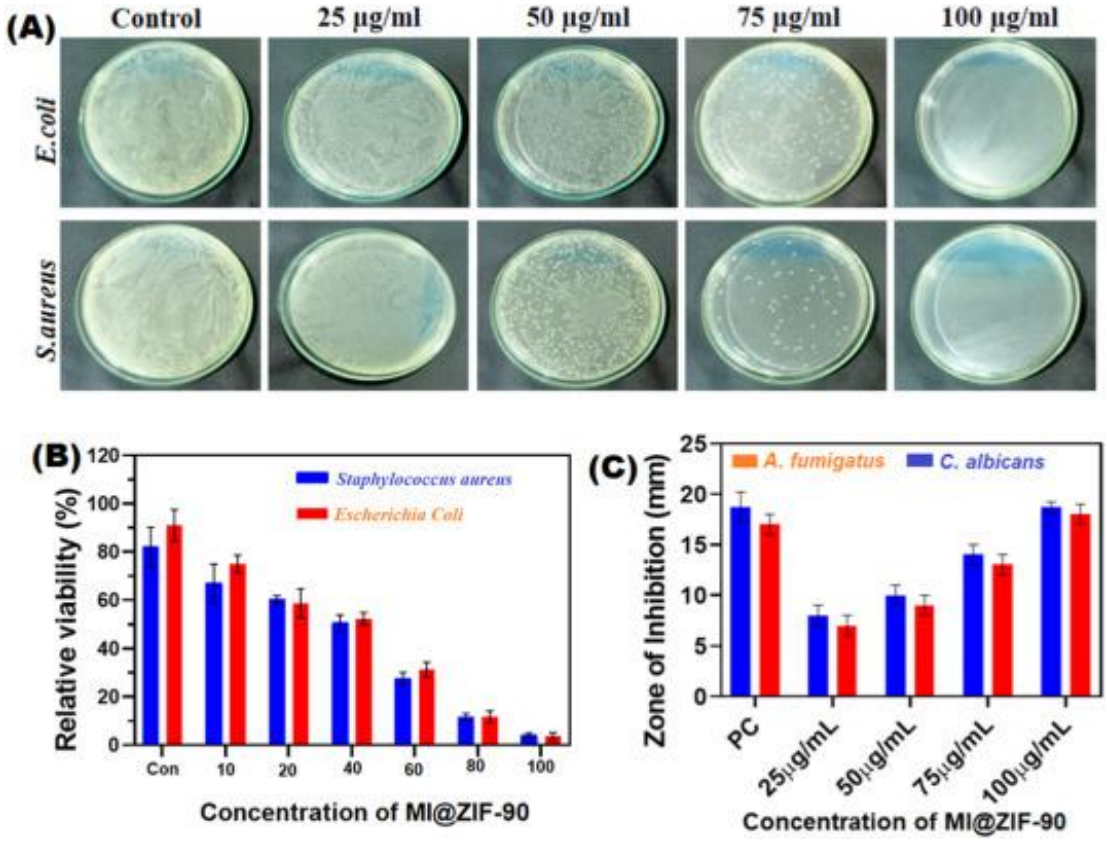

Figure 6: A and B Growth restriction in E.coli and S. aureus cultures treated with MI@ZIF-90. C Diagram of the zone of inhibition in A. fumigatus and C. albicans treated with MI@ZIF-90. Adapted from: Lin T, Qin T, Jiang S, Zhang C, Wang L. Anti-inflammatory and anti-biotic drug metronidazole loaded ZIF-90 nanoparticles as a pH responsive drug delivery system for improved pediatric sepsis management. Microb Pathog. 2023;176:105941. doi:10.1016/j.micpath.2022.105941

6.2.1.3 Nanoparticles in the race against antibiotic resistance

Antibiotic resistance is one of the biggest concerns of the medical community and one of the greatest problems that have to be addressed immediately. Regarding pediatrics the situation does not differ from that of the adults, especially regarding the antibiotics that are widely used such as amoxicillin and cephalosporins. The use of nanoparticles as carriers could achieve to a targeted and prolonged release that could overcome the resistance mechanism of the bacteria and at the same time could hinder the development of new ones[66].

Ceftriaxone is a third generation cephalosporine, used in nearly every specialty for the treatment of a great variety of infections. From meningitis to pneumonia, to other rare infections ceftriaxone is the drug of choice. For pediatrics it also represents a great choice for complicated situations especially regarding the even growing resistance to amoxicillin/ clavulanic acid. It can be administrated from the neonatal age. Sadly, the last years the resistance to ceftriaxone is increasing rapidly.

Duceac et all, proposed the encapsulation of ceftriaxone in a polymeric nanoparticle in order to overcome resistance thru a sustained and targeted release of the drug[66]. The material used was chitosan, which is biocompatible, non-toxic and also possesses antimicrobial properties. This important characteristic of the nanoparticle is achieved firstly by adhering to the bacteria cell wall and modifying its properties and also by inhibiting the replication of the bacterial DNA[66]. Both gram positive and gram-negative bacteria are affected by these mechanisms.

The chitosan- ceftriaxone nanoparticle was studied in order to evaluate its loading capacity, stability and size[66]. The results prove the stability and great loading capacity of the formulation and also calculated its size and release rate of the encapsulated drug, also with adequate results[66]. Although, this sets the ground for more nano-antibiotic combinations, more trials have to be conducted to determine its efficacy and especially its anti-resistance action[66].

6.2.1.4 Taste improvement thru nanotechnology

In pediatrics the administrated medicines are usually either syrups or powders meant to form liquid suspensions. As a result, a lot of medications have not only to be transformed but also to have a child friendly taste. Nanoparticles are of great help as they can encapsule drugs and also form liquid suspensions. A well-known drug praziquantel, which is widely used against parasitic infections faces problems regarding its administration in pediatrics.

Praziquantel (PZQ) is an antiparasitic drug, often used against schistosomiasis. An infection affecting a lot of children under 5 years of age[2]. PZQ is currently available only on tablet form, which hinders the administration in younger patients[2]. In addition to that the metallic taste of the medicine affects the compliance negatively[67,68]. In order to create forms of PZQ suitable for children the medicine is required to have a sufficient aqueous solubility[69]. This is not the case with PZQ, a fact that also affects its bioavailability[69].

To overcome those problems the team of Gonzalez tried to enhance the aqueous solubility of PZQ by increasing its surface area[2]. This was achieved by reducing the size of the particles to nanoscale thru high-pressure homogenization[2]. Although the smaller size enhances the solubility and facilitates the formulation of a different administration form it affects negatively the stability of the system[2]. The freeze drying and spray drying was later used to create stable solid forms to solve this problem. At the end the powder was mixed with a sweet vehicle to improve its taste.[2]

The formation was then evaluated regarding the particle size, morphology, crystallinity and stability[2]. The next step was the assessment of the dissolution and the drug content as well as its taste[2]. The results were satisfactory, giving not only a solution for the treatment of schistosomiasis but also laying the foundation for an easy formation of medications for children[2].

Powder forms are often a better choice in comparison with syrups. They don not usually require a fridge to be stored at after opening, as a result they

can easily be transported and administrated especially in areas with limited resources. Additionally, powders can be dissolved in different liquids such as juices or milk, which improves the compliances.

6.2.1.5 Silver Nanoparticles as antiviral and immunomodulatory agents against RSV infection

Respiratory Syncytial Virus (RSV) is responsible for bronchiolitis in infants and low respiratory infection in older children and also adults especially those older than 65 years of age. The clinical manifestation varies from mild fever with rhinitis to severe illness with need for oxygen therapy. Immunosuppressed patients, premature neonates and infants and those with underlying diseases are at great risk for severe illness. RSV infection accounts for a great majority of hospitalization and deaths of hospitalized children under five years old per year[70]. It is also a leading cause of deaths for infants[70].

RSV is transmitted thru the air and inhalation. It contaminates first the upper respiratory tract and then descents to the lower respiratory tract. Only recently a vaccine has been established, but is not widely available, until then the only weapon against RSV remains the monoclonal antibody which is administrated monthly to infants with high risk for severe disease in case of infecting. Until now the treatment includes mainly supportive means as no effective medication exists. So, the need for one is profound.

Silver Nanoparticles (AgNPs) have shown some promising antiviral properties against influenza, adenovirus and parainfluenza[71–73]. Those nanoparticles owe this property to two mechanisms. First of all, they hinder the entry of the virus to the cell by binding on their on-surface glycoproteins[74]. Secondly, they act inside the cell by prohibiting the virus replication and assembly[73,75]. Morris et al. tested the antiviral and immunomodulatory effect of AgNPs against RSV. The fact that RSV also contains glycoproteins on its surface amplified their assumption[70]. They used A549 cells, which represent human alveolar type II-like epithelial cells, and HEp-2 Cells for their in vitro studies. For the in vivo part of the experiment, they utilized 10 to 12-

week-old BALB/c mice, which were intranasally infected with the virus[72]. The dose of the AgNPs was adjusted according to the weight of each mouse.

The in vitro results demonstrated significant reduction of the replicants of the virus found in the epithelial cells, while the toxicity studies show really low toxicity regardless the number of NPs received by the cells.[72] The in vivo studies confirmed those results by assessing the weight loss, the illness score and examining lung tissue. Additionally, they examined the BALF, where the number of pro- inflammatory cytokines was significantly lower in the AgNPs treated mice than that from the untreated ones[72]. Also, the neutrophil number was higher at the first group, proving that AgNPs practice their anti-RSV effect also by promoting an increase in the neutrophil number[72].

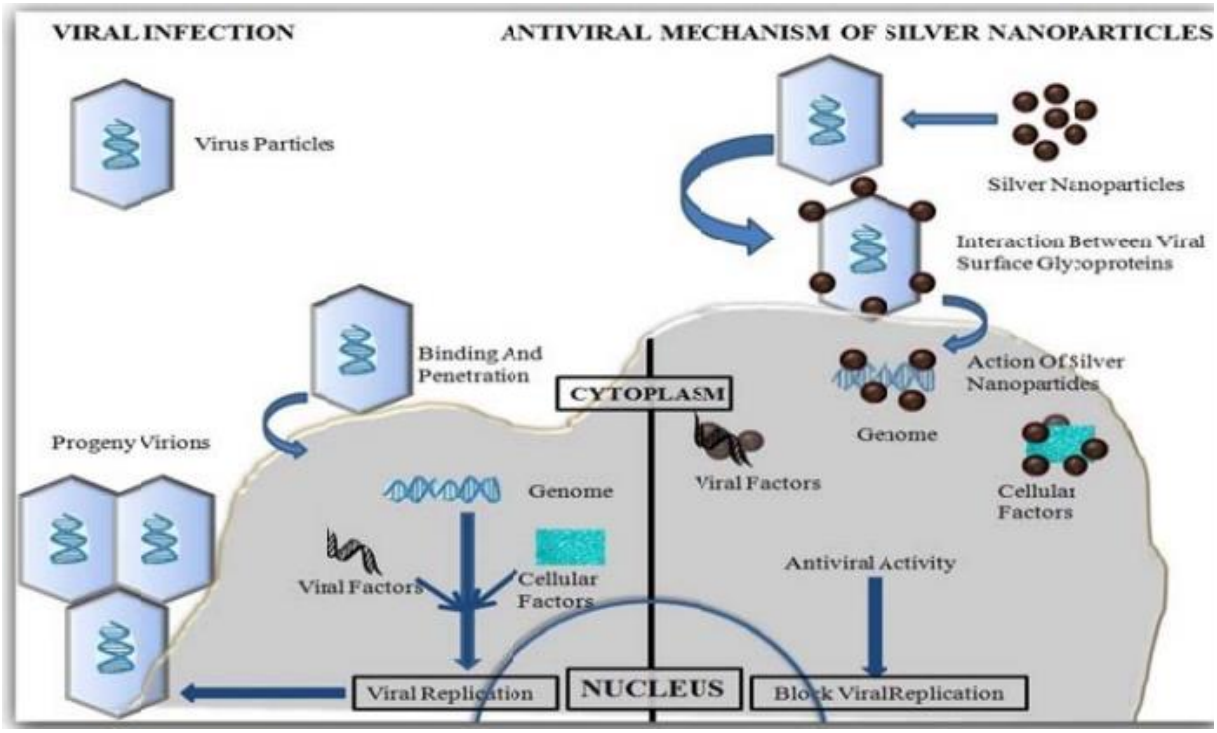

Figure 7: The antiviral properties of silver nanoparticles. Adapted from: Khandelwal, Nitin & Kaur, G. & Kumar, Naveen & Tiwari, A.. (2014). Application of silver nanoparticles in viral inhibition: A new hope for antivirals. Digest Journal of Nanomaterials and Biostructures. 9. 175-186.

6.2.2 Cancer therapy

While nanomedicine provides therapeutic applications to wide variety of specialties and diseases, in pediatrics the most studied is the field of

oncology. According to World Health Organization cancer is the second most common cause of death for children age 5 to 9 years old and the third for them between 9 to 14 years of age. While accidents and suicides take the first place. So, the researcher is strongly aimed at the development of novel and efficient treatments with the less possibly toxicity for the developing organism of the child.

A great variety of anti-cancer treatment options including surgery, chemotherapy, radiotherapy are currently available in pediatric oncology. Nevertheless, due to the unique characteristics of the childhood tumors they are proven less effective against them in comparison with the same types of adult malignancies[76–78]. On the other hand, children fully treated from such malignancies often struggle for the rest of their life due to either side effects of the applied treatments or permanent damage from the tumor and its resection[79]. This includes endocrine imbalance, development and neurological disorders[80].

Nanomedicine provides some very useful tools. Firstly, it facilitates targeted treatments. This can be achieved either by the enhanced permeability and retention effect (EPR) or by creating NPs with special markers to target specific cell populations[3]. The EPR effect is based on the increased permeability of the endothelial cells in cancer tissues that allows the NPs to pass thru them and accumulate inside the tumor microenvironment. On the other hand, cancer cells express a wide variety of unique markers which can be exploited as targets for a more personalized treatment, which is the ultimate goal in oncology[4]. Last but not least is the stimuli responsive treatment a relatively new type, based on the ability of NPs to act under the surveillance of either innate stimulus such as ph and temperature or external such as radiation[81].

Nanomedicine has already improved cancer treatment in adults with a wide variety of different types of therapies. From encapsulation of the very cytotoxic medications, to targeted immunotherapy and radiotherapy the field of oncology has already integrated the use of nano medicinal products in its daily practice with a lot of centers around the world to be using its new applications with great results. The case is not the same with pediatrics where a few trials have only reached the clinical level.

As it has been already mentioned not a lot of clinical trials take place currently in pediatric oncology but there are a lot laboratory experiments with promising results waiting to be test on the clinical field and change entirely the face of pediatric oncology. Some of these interesting discoveries will be discussed on the paragraphs below.

Central nervous system (CNS) malignancies represent the majority of solid tumors in childhood cancer. As a result, most of the applications mentioned below refer to their treatment. Additionally, the BBB creates a significant obstacle to the entrance of the medicines in the brain. BBB consist of astrocytes, pericytes and endothelial cells, which combined with the tight junction formed between them create an impermeable membrane.

The entrance is mainly achieved thru pumps activated by a variety of receptors such as transferrin and insulin. Hydrophilicity and size also control the entrance of substances[82]. The hydrophobic and small molecules are the ones able to cross it. Nanoparticles provide as small size and the hydrophobic structure; they can also be formulated with the suited markers one their surface to bind to the pumps[82].

CNS malignancies in pediatric population differ greatly from those of the adults. Firstly, the heterogenicity of the cancerous cells complicates a lot the treatment process[77]. In addition, the presence of the cancer stem cells (CSCs), a recently identified moiety creates an even greater challenge for the medical community [77]. CSCs represent a cell population characterized by the ability to self-renew, adapt to harsh conditions, diversify and create tumors[77]. Such cells have been identified in some CNS malignancies like medulloblastoma, ependymoma and gliomas[8378]. Their presence has been linked to worse outcome with decreased response to both radio and chemotherapy[84,85].

In addition, some aggressive types of brain cancers develop mechanisms to overcome both the applied treatments and the natural apoptotic pathways[86]. By modulating the membrane pumps they remove the anticancer drugs and by modifying the apoptotic pathways escape death[86]. As a result, those cancer populations survive therapies and worsen the outcome.

6.2.2.1 Gene therapies

Gene therapy refers to the modification of the cell response in genetic level by administrating the appropriate nucleic acid. Nucleic acids are rapidly dissolved when entering the systematic circulation, hence the need for a transporter. In the past viral transporters have been tested some of them with promising results[87]. Nevertheless, nanoparticles offer alternative carriers with the advantage of the special properties that it's one of them provide.

6.2.2.1.1 Small interference RNA (siRNA)

During the creation and development of a malignant cell population a great variety of signaling pathways are activated and at the same time the repairing and apoptotic mechanisms are being suppressed or deactivated[76]. The modification of those in gene level could significantly improve the therapeutic outcome. A great obstacle to these types of therapy is the instability of the nucleic acids and especially regarding CNS tumors the low permeability of the BBB.

The encapsulation of the nucleic acid in nanoparticles could give a solution to this problem, while the functionalization of their membrane with the proper molecules would lead to a more targeted treatment and also overcome the BBB[76]. Regarding cancer treatment siRNA is mainly used in order to silence a variety of genes, leading to the modification of signaling pathways[76].

Liu et all designed a gold nanoparticle, which would encapsule siRNA against Ape 1 gene to treat Ependymoma and Medulloblastoma in children[13,76]. Ape 1 the gene responsible for the expression of an enzyme that repairs the damage caused by the radiotherapy[13,76]. The silencing of such a gene would increase the sensitivity of the tumor to radiotherapy[13]. The engineered AuNPs where coated with polyethylene glycol (PEG) and contained positive charged polyethyleneimine which assisted with the binding of the negative charged nucleic acid. At the same time chitosan enabled the covalent binding of them with the NPs[13].

The results indicated stable binding of the siRNA, while transfer and uptake were also successful. The silencing of the gene led to lower expression of the Ape 1 and combined with γ-irradiation had the desired result, with significant damage to the DNA of the cancer cells and activation of the apoptotic mechanisms[13]. Kievit et all also created a nanoparticle with superparamagnetic iron oxide core and coating with chitosan, polyethylene glycol and polyethyleneimine to treat medulloblastoma by suppressing the Ape 1 gene with very good results[12]. Both studies hold great promises for the until now radio resistant type of cancers[13].

Another promising formulation of LNPs- siRNA was designed to treat acute myeloid leukemia (AML)[88]. Regarding AML the prognosis is usually poor for young patients mainly due to the toxicity both of the chemotherapy and the more conventional CAR-T cell therapies[88]. In addition to that the lack of adequate information regarding the pathophysiology behind AML hinders the creation of an effective drug. Recently light was set to the role of the non-coding genes and RNAs to the regulation of the gene expression[88]. Such an area was lately found in the t (8;21) translocation[88].

Connerty et all, identified the exact non-coding sequence LINC01257 and investigated its correlation with AML, to prove its oncogenic profile and relation with worst prognosis and poorer therapeutic outcome[89]. On this ground the team designed a formulation, which consisted of an LNP as a vector and a siRNA to target the long non-coding RNA (lncRNA) region LINC01257. LNPs represent the most well-known and tested vehicles for nucleic acid transfer[89].

Cell cultures from children with AML were used for the purpose of the study. The results proved the oncogenic lncRNA LINC01257 and its specificity for AML, due to the fact that it was not expressed in healthy tissues, and the positive effect of its silencing in the survival of the cells cultures. Additionally, the formulation achieved impressive uptake rate and the no significant toxic was observed to the healthy cells.[89] Nevertheless, more clinical trials in animal models have to be conducted to evaluate the results thoroughly.

6.2.2.1.2 Micro RNA (miRNA)

MiRNA represent a type of non-coding RNA found in pediatric brain tumor cells[90]. They play a key role in the response to chemotherapy and in the behavior of the tumor in general[90]. They work as epigenetic modulators for mRNAs promoting oncogenesis, while suppressing the natural anticancer mechanisms[91]. Additionally, they can be associated with the anatomical location of the tumor[92]. Such miRNAs have also been found in cancer stem cells[93]. Their deregulation is proposed as a factor that affects the progression of the tumor[93]. As a result those miRNAs provide a new therapeutic target.

Lopez-Bertoni et all developed a polymeric NPs as carries for miRNA to treat GBM[91]. To optimize the binding of the nucleic acid they opted for the cationic Poly(beta-amino ester)s (PBAEs)[76,91]. PBAEs not only are able to encapsule efficiently the miRNA but are also bio reducible. In addition, PBAEs provide a triggered release as soon as it enters the cytosol, while protecting the miRNA from the endosome[91]. The NPs contained either one type of miRNA or a combination of them[91]. They constitute mimics of the miRNAs of the CSCs leading to a reduced development of the CSCs of the BGM[91].

The results show a reduction of the growth of the orthotopic human GBM xenografts[91]. Especially when combined with γ-radiation the response was significantly increased[91]. At the same time to toxicity was subtle. This study provides a great example on how the appropriate NP can facilitate different types of miRNAs to treat not only types of cancers but also other diseases, especially those related to a genetic disfunction[91].

6.2.2.1.3 DNA therapies

Poly (beta-amino ester) nanoparticles (PBAE) represent a conventional and promising nanocarrier for nucleic acids[87]. They exhibited great results when testing in adults for cancer treatments with minimum side effects[87]. At the same time, they prove to having a really good immunogenic profile making them suitable candidate for treatment of childhood cancers. They are polymeric

nanoparticles with high ability to encapsulate DNA and the transfer it in inside the cell avoiding the endosome and leading to the expression of the carried genes[94]. Additionally, their rapid degradation decreases their potential toxic effect to the growing organism of the child[87].

Choi et all. based on the positive results received from the encapsulation of the DNA responsible for the encoding of the thymidine kinase (HSVtk) of the simplex virus type I at the adult glioma constructed a PBAE NP to transfer the same DNA to treat atypical teratoid/rhabdoid tumors and medulloblastoma at athymic mice[95]. Teratoid/ rhabdoid tumors represent the commonest type o brain tumor at infants younger than 6 months of age[87,96]. Their conventional treatments have significant impact to the development of those children.

The plasmid DNA was encapsulated effectively due the presence of hydrolytically cleavable ester bonds[94]. Different types of polymers were used to determine which one of them of them has the better results in its type of tumor[96]. At the beginning the NPs uptake was tested in vitro in cell cultures and later ganciclovir was added[96]. When tested in vivo the NPs were administrate by conventional enhanced delivery, while for ganciclovir they opted for the intraperitoneal one[96].

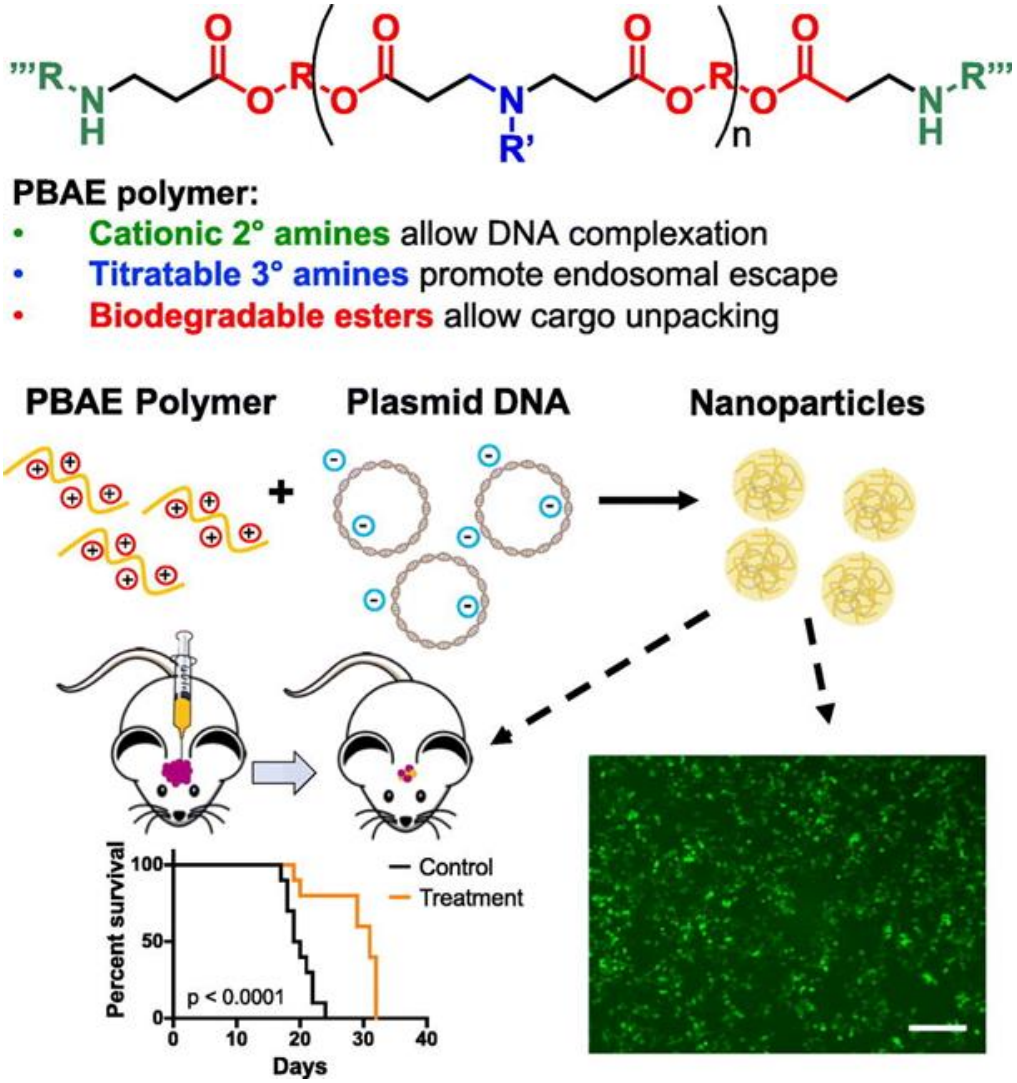

Figure 8: Schematic illustration of the encapsulation of the plasmid DNA in the nanoparticles and the intracranial administration to the mice. Adapted from: Choi J, Rui Y, Kim J, et al. Nonviral polymeric nanoparticles for gene therapy in pediatric CNS malignancies. Nanomedicine. 2020;23:102115. doi:10.1016/j.nano.2019.102115

The result revealed significant increase at the survival of the treated population[96]s. A very interesting observation was that even minimum differences at the NPs structures can affect the uptake and transfection process, while its type of polymer presented better results for different tumors[87]. Although PBAE are very promising as DNA vectors for gene treatments in pediatric cancers, with low toxicity and side effects, more studies have to be conducted to determine the uptake mechanisms and pathways[96].

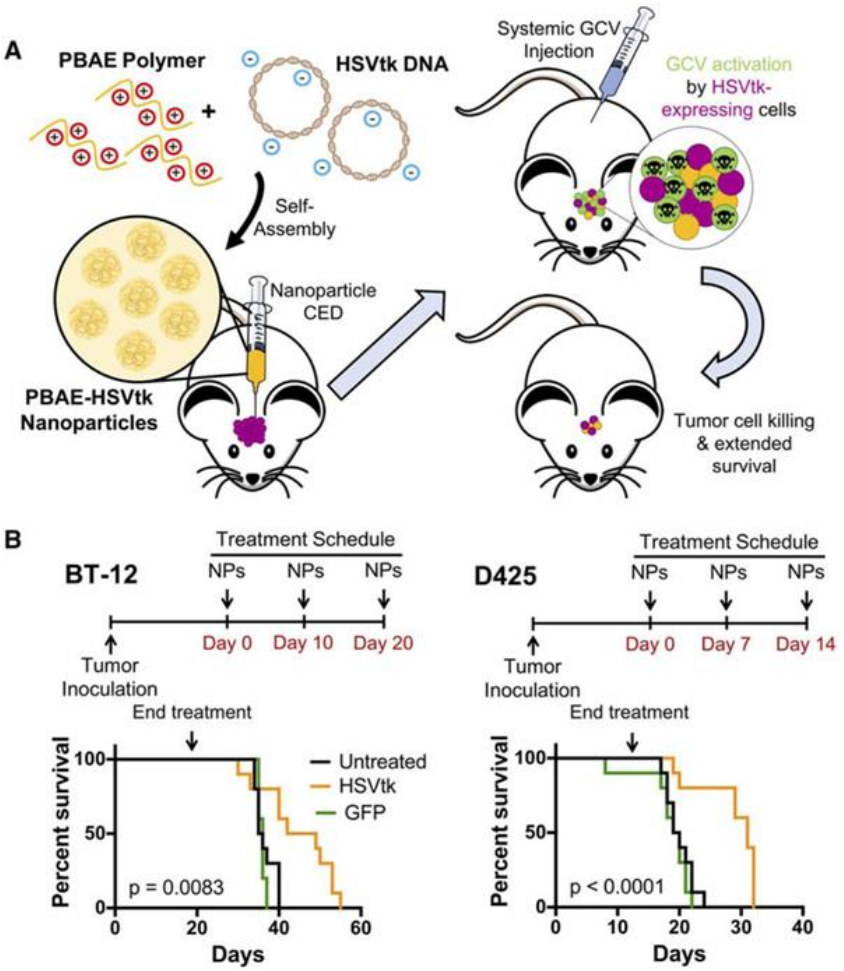

Figure 9: Graphic depiction of the experiment procedure, with the survival result charts at the end. Adapted from: Choi J, Rui Y, Kim J, et al. Nonviral polymeric nanoparticles for gene therapy in pediatric CNS malignancies. Nanomedicine. 2020;23:102115. doi:10.1016/j.nano.2019.102115.

6.2.2.2 Immunotherapy

Malignant cells in order to survive and spread around the body are able to either deactivate the immune system or to hide from it by expressing marker found in healthy cells[23]. Immunotherapy refers to the redirection of the immune system against the tumor and its microenvironment[23]. That can be achieved either by reactivating the immunity mechanisms or by targeting those specific cells using antigens on their surface[23]. Nanoparticles provide perfect vehicles to safely transfer and specifically target those cells.

Children's immune system differs greatly from those of the adults. From birth until adulthood it changes, going thru different stages, which vary greatly both in functionality and maturity, hence a lot of the immunotherapies already

designed and approved for the adult patients hack efficiency in pediatric population and the necessity for new ones tailored for the developing immune system of the young patients[23].

In this ground Mendez-Gomez et all designed a lipid nanoparticle able to carry different kinds of mRNAs to treat pediatric gliomas[97]. It contained specific 5' and 3' untranslated regions, that were able to incorporate either personalized mRNA or the H3K27M mRNA. The results show significant anti-tumor response with longer survival rates in murine brain tumors. One of the most interesting aspects of this study was the ability to include also other mRNAs for co-delivery. Toxicity studies proved its safety leading to obtainment of FDA- IND approval for human trials in pediatric population with high- grade gliomas[23,95].

To target a specific cancer cell population and create an immune response against it, it is necessary to find markers expressed only in those. Otherwise, the healthy cells will also be targeted and the immune response will be massive creating an inflammatory environment and causing damage to even healthy tissues[23]. The stimulator of the interferon genes (STING) represents a pathway expressed greatly in cancer tissues and especially in neuroblastoma cells[81]. This offers the opportunity to activate the intrinsic immune mechanisms against the tumors and its microenvironment, creating a T -cell mediated response that could lead to cell death[97].

Neuroblastoma represents the third most common type of malignancy in pediatric population, but nevertheless one of the most aggressive[97]. Most of the already applied therapy regimes have poor results with really high relapse levels[97]. Most immunotherapies applied in adult patients does not have the same result in children. Neuroblastoma is characterized by a highly immunosuppressed population of cells, while the infiltration of T-cells is really low[97].

The team of Wang-Bishop exploited the STING pathway to treat neuroblastoma in mouse models[97]. To active it they utilized the 2'3'-cGAMP, which is a natural ligand and presents with high affinity for the pathway[97]. As vehicle the used a polymersome coated with PEG to enter the 2'3'-cGAMP inside the cell. When the

NP enters the endosome due to the low ph of the last the polymersome disassembles while at the same time mediates the transfer of the 2'3'-cGAMP to the cytosol[97].

The NPs were directly administrated inside the tumors. The activation of the STING pathway was tested in neuroblastoma both with and without N-MYC amplification with similar results[97]. In both the activation of type 1 interferon and other proinflammatory cytokines was massive, while the concentration of T-cells in the cancerous microenvironment was increased[97]. In addition to that, cell death was also activated leading to the recession of the tumor growth[97]. A very interesting aspect was that immunological memory was also created, which protected from a potential relapse[97]. This opens the road for immunotherapy in children for this very aggressive type of malignancy[97].

6.2.2.3 Drugs

The most traditional load of NPs are conventional drugs, which constitute the active ingredient of the final medicinal product. In most of the cases the active ingredient shows high toxicity with systematic side effects when administrated alone. Additionally, most of the anticancer drugs are unable to cross the BBB. NPs utilize receptor mediated endocytosis to achieve the entrance inside the brain. Insulin, transferrin and endothelial growth factor are the most common[23,76].

Furthermore, there are drugs, which exhibit great results regarding their efficiency against cancer cells but lack in pharmacokinetics. In detail they either degrade rapidly when entering the body or lack stability[5]. As a result, the amount of the medication that reaches the tumor is really low in comparison with the administrated, leading to an unnecessary exposure only to the side effects with minimum anticancer effect. This is the case with a derivative of temozolomide (TMZ) N(3)-propargyl, a great candidate against diffuse intrinsic pontine glioma (DIPG) in pediatric population[5].

DIPG represent a very aggressive and common (75% of the gliomas) type of pediatric cancer with resistant to the well-known TMZ used in adults[5]. At the same time, due to its location the option for dissection does not exist leading to a very bad prognosis. As a result, from studying the mechanisms responsible for the resistance to TMZ, occurs that the derivative of temozolomide (TMZ) N(3)-propargyl could bypass them and act against the cancer cells. To overcome the pharmacokinetic obstacle mentioned above two NPs were proposed.

The first one is an apoferritin (AFt) nanocage, which targets the abundant transferrin receptor 1 found on the cancer cells, while its ph dependency allows the release of its content only inside the lower pH of the malignant microenvironment[5]. The second one included a sulfobutyl ether β-cyclodextrin

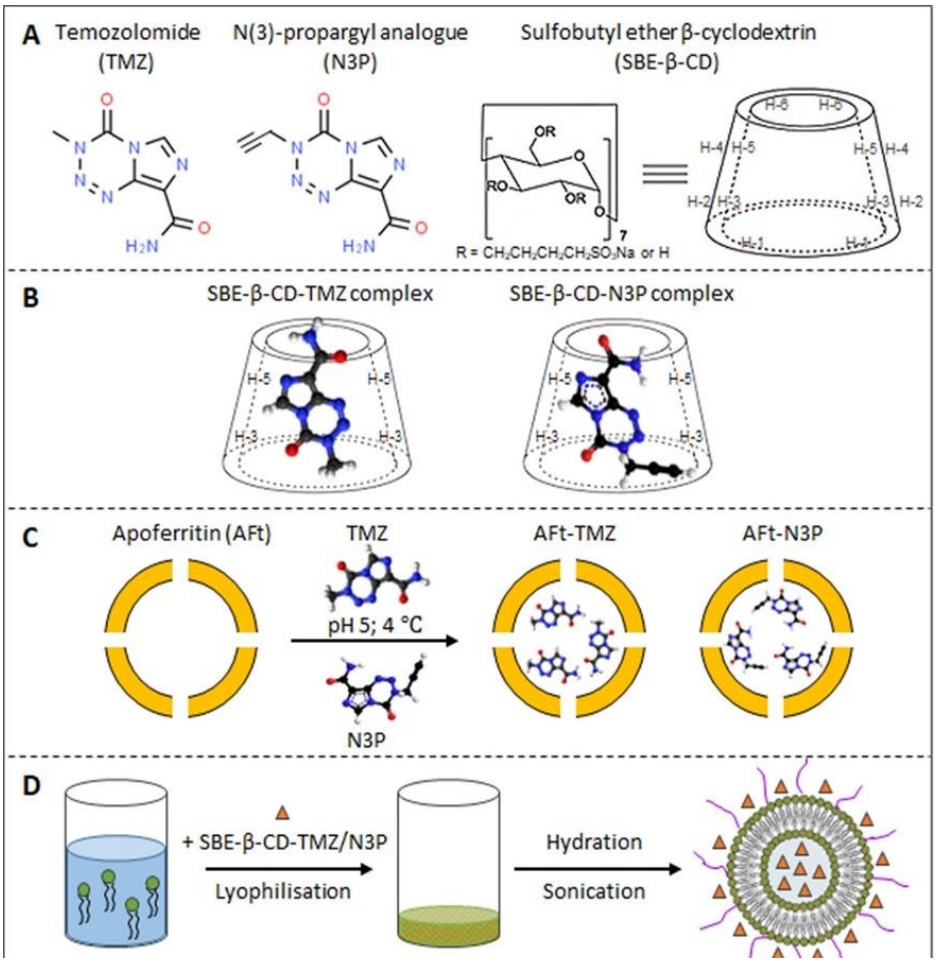

Figure 10: Illustration of the components and the encapsulation procedure to form the SBE-β-CD-TMZ/N3P complexes and conversion into nanoliposomes. Adapted from: Heravi Shargh V, Luckett J, Bouzinab K, et al. Chemosensitization of Temozolomide-Resistant Pediatric Diffuse Midline Glioma Using Potent Nanoencapsulated Forms of a N(3)-Propargyl Analogue. ACS Appl Mater Interfaces. 2021;13(30):35266-35280. doi:10.1021/acsami.1c04164

complex transferred inside a liposome[5]. Cyclodextrin was used in order to enhance the encapsulation of insoluble drugs into the aqueous core of the liposome and to prevent both leakage of the drug and its interaction with the membrane[5].

Both complexes achieved good loading and were administrated with convection-enhanced delivery direct to the brain of the rats bypassing the BBB. Both provided good pharmacokinetics with sustained release and stability. Especially, the exploitation of transferrin receptor 1 as a target lead to great amount of drug entering the tumor cells. The combination of NPs and convection-enhanced delivery could provide great solution for the treatment of a variety of intracranial moieties, but the need for more preclinical trials to fully understand its mechanisms is imperative[5].

Hydrophilicity and lipophilicity pose another significant obstacle NPs can easily overcome. They can be manufactured to change their solubility depending on the environment. In this ground Infante et all developed a micelle loaded with Glabrescione B to treat H-dependent medulloblastoma (MB)[98]. Glabrescione B is a hedgehog inhibitor with poor water solubility, which affects negatively its pharmacokinetics[98]. The micelle is amphiphilic self-assembled and improves pharmacokinetics and solubility of the complex, which displayed minimum toxicity[98]. The administration of the medicine resulted in a significant decrease at the growth of the tumor[98].

On the other hand, NPs provide the ability to combine drugs for a better synergistic result. A great example was designed by Yang et all to treat neuroblastoma in mouse models[99]. A liposome was the vehicle loaded with picoplatin (IV) and cantharidin. Picoplatin is a very strong chemotherapeutic agent, but is only active in its II form, which is also extremely toxic. Hence it was encapsuled in its IV form to be converted inside ta cancer tissue. The results indicated a significant reduction in the tumor size, with really low levels of toxicity[99]. Additionally, this co-administration did not only overcome the chemoresistance but also gave the opportunity to have similar results with lower doses of the drugs[81].

6.2.2.4 Nanoparticles for targeted therapy in brain cancers

Piumi Y. Liyanage et. all. synthesized a nanoparticle containing Gemcitabine (GM) and transferrin protein (Tf)[11]. Carbon Nitrate dots were used as nanoparticle materials due to their water solubility, low-toxicity, biocompatibility, high photoluminescence and their adjustable structure[100,101]. Gemcitabine an anti-cancer agent, leading to cell death by inhibiting the synthesis of the DNA thru DNA polymerase, whose activity is being blocked[11]. Radio sensitizing ability has also been mentioned, making it a perfect candidate for GBM treatment[102]. In order to overcome the BBB, the team utilized the Tf, to take advantage of the receptor-mediated endocytosis[103]. In addition to that, Tf receptors are abundant in cancer cells, enabling a more targeted therapy[104].

Firstly, the Carbon Nitrate Dots (CNDs) were synthesized and the conjugated with gemcitabine and later with the transferrin, this was performed by the creation carbodiimide crosslinking[11]. Then the nanoparticles were characterized in order to define their physicochemical properties and ensure the attachment of both GM and Tf to CNDs. The next step of the experiment focused on the efficacy and anticancer effect of the medicinal product by testing GM alone and GM-CNDs. The results were positive, but resulted also to severe damage of the neighboring cells. To overcome this the targeting ability of Tf receptors was utilized. The CNDs-GM-Tf resulted in the elimination of only the cancerous tissue leaving the healthy unharmed. So, the targeting capability and the anticancer effect of the system was established[11].

The next step was to ascertain the ability of both the CNDs alone and the conjugated form to cross the BBB. This part of the experiment took place in zebrafish, a perfect candidate for this purpose. CNDs achieved sufficient enter in the brain. The team hypothesized that this occurred due to their small size and specific structure. An additional hypothesis to explain it, was thru a transporter-mediated channel, by mimicking the glutamine structure. The CNDs-GM was tested next also with very good results. At the end the CNDs-GM-Tf displayed the best results among the three, proving that the Tf not only enhance a more targeted treatment but also

improves the BBB crossing and reassures that the medicine reaches the pathological cells[11].

6.2.2.5 Multidrug Nanoassemblies

A very interesting formulation was engineered by Rodríguez-Nogales et all[105]. In order to by-pass the skepticism around the use of nanoparticles as vector for medicines in pediatric cancer therapy and also the loading and release problems the team created a nanomedicine only be combining two already known drugs with squalenic acid[106]. The last one represents a lipid molecule with no toxicity and the ability to self-assemble when connected with the drug in order to create stable nanostructures[106].

The active substances that were used were Gemcitabine and Edelfosine. Gemcitabine is an anticancer nucleoside analogue with amphiphilic structure, which use is restricted due to its fast degradation and developing resistance[107]. Edelfosine is an alkyl-lyso-phospholipid anticancer agent with also an amphiphilic structure[105,106]. This last common characteristic of the two drugs was necessary for the formation of the nano-assemblies, which took place thru the nanoprecipitation method. Dynamic light scattering combined with cryo-Transmission electron microscopy contributed to the characterization of the nanoformulation[106].

The anticancer effect of the multidrug was later tested on cell cultures derived from osteosarcoma metastasis of pediatric patients[105]. At the same time its efficacy was compared with the one of the two drugs alone. The results proved the superiority of the multidrug, its anticancer effect which was reinforced due to the synergistic effect of the two drugs. The formulation also displayed its anticancer effect at lower concentration of its compound lead to an also lower toxicity[105].

Later the pharmacokinetics were investigated in vivo in mice[105]. The intravenous administration was successfully achieved without the hemolytic effect of edelfosine at the same time the formulation led to a sustained release of

gemcitabine decreasing significantly its toxicity. As result, these multidrug nanoassemblies provide a great alternative for the treatment of aggressive pediatric metastatic cancers and also sets the ground for more multidrug nanostructures[105].

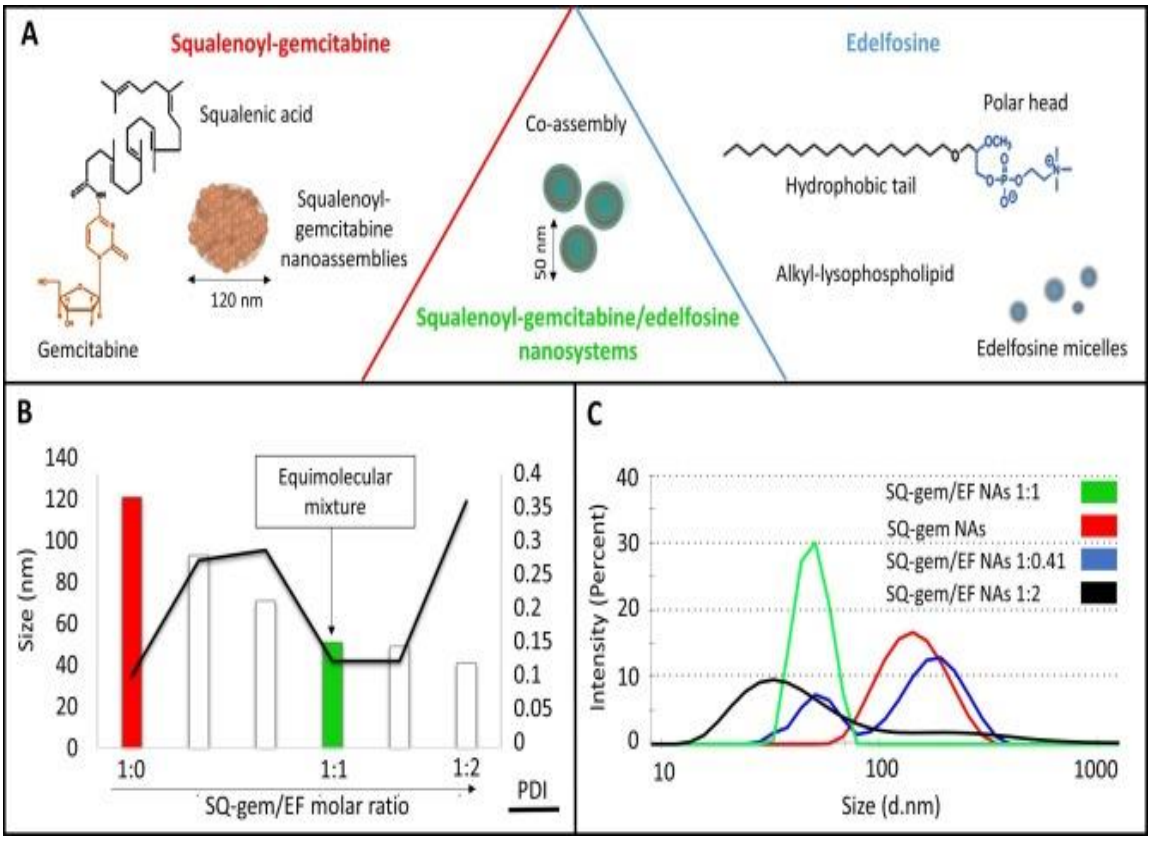

Figure 11: Diagram of the nanoparticle formation, with charts about their size in correlation with the concentration of each component of the NP. Adapted from: Rodríguez-Nogales C, Sebastián V, Irusta S, Desmaële D, Couvreur P, Blanco-Prieto MJ. A unique multidrug nanomedicine made of squalenoyl-gemcitabine and alkyl-lysophospholipid edelfosine. Eur J Pharm Biopharm. 2019;144:165-173. doi:10.1016/j.ejpb.2019.09.017

6.2.2.6 Nanoparticles as sensitizers for radiotherapy and photothermal therapy

Nanoparticles are already used either as sensitizers or as vehicles for them to treat a great variety of cancers in adult patients. While the concept of radiotherapy is widely known and used this of photothermal therapy represent a conventional method of applying heat and light to a nanodevice in order to promote the death of the cancer cells or the expression of specific antigens and markers[81]. This last effect of photothermal therapy can be exploited as potentional targets for immunotherapy[81].

Gold nanoparticles (AuNPs) stand out in radiotherapy, since they amplify the damage in DNA and inhibit angiogenesis as a result of the applied ionizing radiation[108]. Peg coated AuNPs were used to treat glioblastoma multiform in mice models[108]. When combined with radiotherapy they increased the survival of the models[108]. Silver Nanoparticles also present some radio sensitizing properties against gliomas[23].

Regarding photothermal therapy once again AuNPs play a key role, due to their high light absorption and ability to be tuned[23]. Gold Nanoparticles functionalized with arginine– glycine–aspartate peptide or epidermal growth factor on their surface present a targeted approach to treat glioblastoma in combination with photothermal therapy[23].

A very interesting application waw introduced by Cano-Mejia et all, where Prussian blue photothermal therapy (PTT) was combined with immunotherapy against neuroblastoma in mouse models[14]. Prussian blue is an already FDA approved medication, including pediatric population, against radioactive poisoning[14]. The engineered Prussian blue NPs (PBNPs) present with both photothermal and pH depended properties[14].

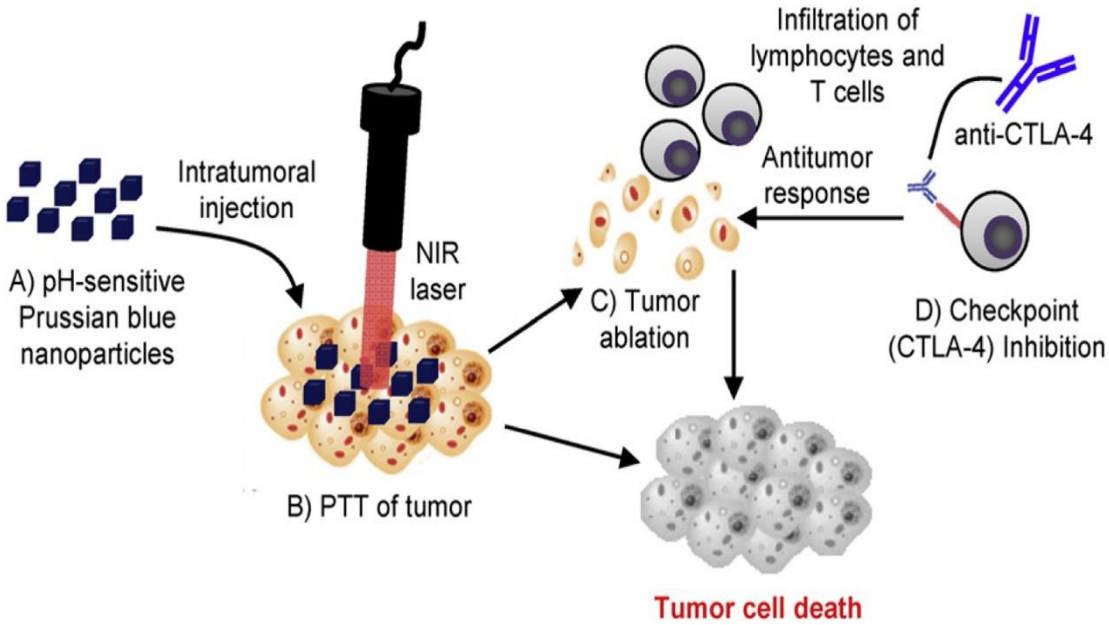

Figure 12: Schematic illustration of the combination of PTT and immunotherapy against neuroblastoma. Adapted from: Cano-Mejia J, Burga RA, Sweeney EE, et al. Prussian blue nanoparticle-based photothermal therapy combined with checkpoint inhibition for photothermal immunotherapy of neuroblastoma. Nanomedicine. 2017;13(2):771-781. doi:10.1016/j.nano.2016.10.015

The administration took place during the operation, which is the most widely used therapy for neuroblastoma[14]. The pH dependency ensured the stability of the PBNPs inside the tumor environment and at the same time its degradation immediately after entering the blood circulation or the lymphatic system causing minimizing the toxicity[14]. PTT was applied thru a near infrared laser.

Immunotherapy was applied on day 1,4 and 7 simultaneously with PTT and alone one days 0,3 and 6[14]. Anti-CTLA-4 antibody represented the immunotherapy agent was in this study. The results show not only the complete regression of the tumor mass but also great survival rates when combined to either BPNPs-PTT or immunotherapy alone[14]. Creating the opportunity to farther investigate the combination of PTT with immunotherapies for better outcomes in cancer therapy.

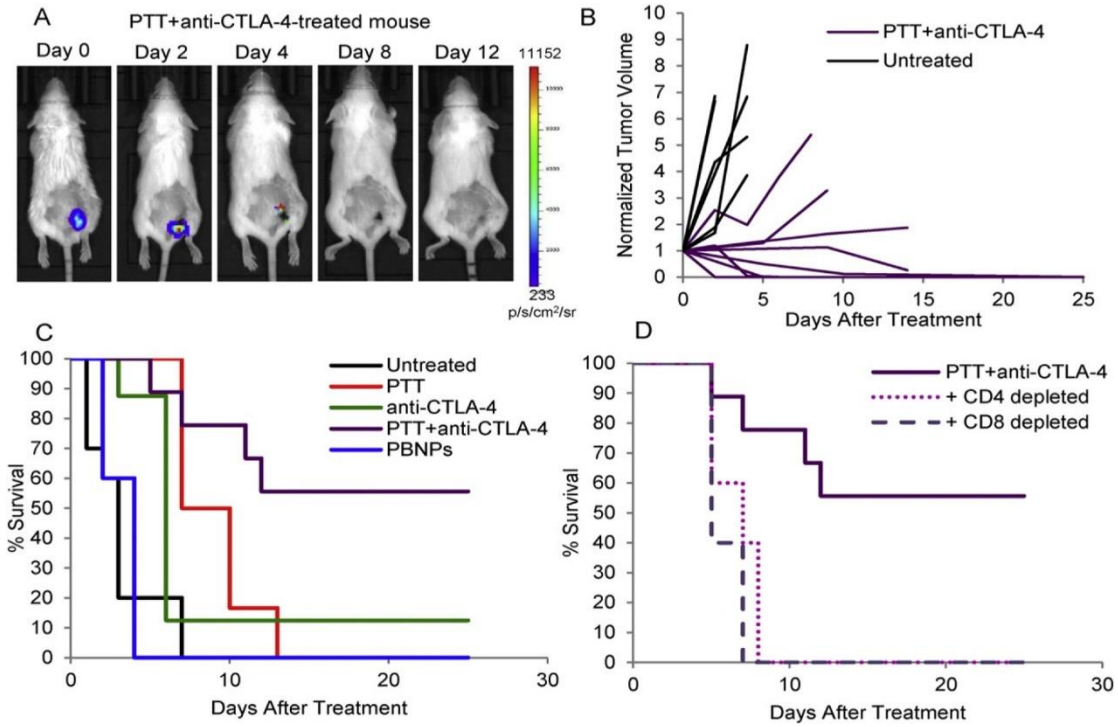

Figure 13:A Depiction of the tumor growth restriction after the therapy was applied, B, C charts of the comparison of the survival rates during the experiment and the progression after every treatment. D chart of the CD4+ and CD8+ depletion after PTT. Adapted from: Cano-Mejia J, Burga RA, Sweeney EE, et al. Prussian blue nanoparticle-based photothermal therapy combined with checkpoint inhibition for photothermal immunotherapy of neuroblastoma. Nanomedicine. 2017;13(2):771-781. doi:10.1016/j.nano.2016.10.015.

6.3 Other applications

6.3.1 Nanoparticles as tools for the research in Pediatric

Nanoparticles possess very important features; they can be modified to target specific cells. This can be achieved with a great variety of mechanisms. Antigen- antibody is well known one already broadly exploited one. A relatively novel strategy utilizes cell membrane receptors to target specific cell populations[11]. Nanoparticles can be formulated as to express those molecules able to enter the cells using this receptor mediated mechanism.

This strategy enables the research regarding the expression of specific markers or receptors on pathological cells, so as to either create targeted treatments or to pathologies behind specific diseases. Irregular expression of membrane ligands is very often the result of gene mutations or different needs of the cell that can further lead to the root of the disfunction and subsequently to potential treatments[109]. In addition, the efficacy of the already existing drugs against cancer population is often test by using NPs as their carriers.

On this ground, Pascual-Pasto et all designed albumin bound nanoparticles and encapsulated paclitaxel (nab-paclitaxel) in them in order to study both the accumulation of the drug inside the Ewing sarcoma cells and its efficacy[109]. The experiment was based on the overexpression of the secreted protein acidic and rich in cysteine (SPARC) in a lot of cancer types and especially in sarcomas. SPARC is found on the membrane of the cells and presents with high affinity with albumin.

Firstly, the characterized the relevance between the expression of the SPARC and gene mutations found in pediatric Ewing sarcoma, rhabdomyosarcoma and osteosarcoma xenograft[109]s. Then, the accumulation of nab-paclitaxel and soluble paclitaxel and docetaxel between Ewing sarcoma cells with and without the overexpression of SPARC was compared. Nab-paclitaxel displayed longer accumulation at the ones with the higher expression of SPARC on their membrane. On this ground they will proceed to the next phase of their experiment in order to

determine the use of SPARC expression as biomarker for nab -paclitaxel efficacy against Ewing sarcoma[109].

7 Discussion

Nanoparticles have emerged as a beacon of hope for a lot of specialties including pediatrics. Their applications vary greatly as they can improve and evolve nearly every type of treatment and diagnostic procedure. Their small size and unique properties open new horizons in medicine and pharmacy.

A great many of neurological and metabolic disorders, which today are untreatable, may find a cure thru the utilization of nanomedicine. The small size can facilitate the entrance inside the brain. The controlled release could ensure that the drug could act at a specific environment and minimize the side effects. Gene therapies can also be widely used with nanoparticles as carriers for the nucleic acids[76,90].

Biosensors have gained a lot of confidence in the last years as they speed up the diagnostic procedure and at the same time lower their cost[6,7,48]. Their sensitivity has improved in a great level since the integration of nanoparticles[6]. Especially the gold nanoparticles, which are perfect candidates for those applications[6,7,48]. An additional advantage of biosensor is the sample collection, which is usually easier and provide results faster with a great sensitivity and specificity[6]. Therefore, the manufacture of sensor and point of care test has a lot of potential in pediatrics.

Imaging in pediatrics faces two great problems firstly the significant effect of radiation to the developing organism and the side effects of the contrast agents. Secondly most of the imaging techniques require not only the cooperation of the patient but also to be completely stable for a great amount of time. Children tend to receive anesthesia to undergo those tests. By using Ferumoxitol as contrast agent the longer half life time enables the feed and sleep technique to be applied[8,9]. This novel contrast agent has proven safety, less side effects that the already used one

and also provides better imaging results. Although, ferumoxytol is already approved as an iron supplement it has not received an approval as a contrast agent yet. Both pediatricians and radiologists have to be trained properly to be able to incorporate it at the daily basis.

Oncology has been the center of attention regarding nanoparticles in pediatrics. Although the number of the patients is smaller than those in adults the side effects from the applied radiation and medication are of great importance. They can have a negative effect to the developing organism. Nanoparticles can work as carries for nucleic acids that modify the tumor properties in various ways[3,4,23]. They also act as radio sensitizers reducing significantly the amount of radiation a child receives, which is an important achievement in pediatric oncology[14,108]. Additionally, by providing a targeted and modified release of the already known anticancer drugs they minimize their side effects, leading to a better quality of life[23].

Although, nanoparticles have a lot of potential in pediatrics, the research faces some important obstacles. First of all, is the lack of pediatric models for the in vivo testing of the products[15]. The development of such models could significantly enhance research in pediatrics. Regarding the clinical trials and limitation in pediatric oncology.

On the other hand, nanomedicine is a relatively new domain, especially for pediatrics. The skepticism around it is another obstacle for the researchers as it hinders the clinical stages of the trials. This hesitation, which involves not only the parents but also the clinicians, can be overcome thru safety trials, which would prove the low to non-toxicity and biocompatibility of the nanoparticles.

8 Conclusion

Although a lot of research around nanoparticles in pediatrics exist, they have not been introduced widely in pediatric diagnosis and therapy yet. Nevertheless, a lot of applications are under investigation. Most of them have not reached the clinical trial level yet. Nanocarriers can assist at the creation of child friendly forms, that would easily be administrated to the young patients at the appropriate dose[1]. A more personalized approach will be feasible due to the opportunities that targeted and gene therapies provide[5,76,87]. Ferumoxytol can improve the diagnostic procedure in every aspect. [8–10,17–19,21,33,37,43,45,46]

Although the majority of research is currently focused in oncology the combination of the special characteristics of the nanoparticles, like targeted release, with already known and used antibiotics or antiviral drugs has a lot of potential. Additionally, nanoparticles could act as carriers for a better administration of medications such as insulin and for gene therapies for early intervention in genetic disorders. In conclusion, the future of nanomedicine in pediatrics differs greatly than the one in other specialties and important steps have to be taken towards to infectious and genetic diseases.

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