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HLA Genotyping using Next Generation Sequencing

C. LUCAN[1*], LAURA-ANCUTA POP[1*], A. FLORIAN[1*], VALENTINA PILECZKI[1], B. PETRUSHEV[1], DELIA DIMA[2], IOANA FRINC[2], IOANA BERINDAN-NEAGOE[3], A. IRIMIE[4], C. BERCE[1], I.-S. FLORIAN[1], ANCA BOJAN[1,2], C. TOMULEASA[1,2]
[1]"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Department of Hematology, "Ion Chiricuţă" Oncology Institute, Cluj-Napoca, Romania
[3]"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
Department of Functional Genomics, "Ion Chiricuţă" Oncology Institute, Cluj-Napoca, Romania
[4]"Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
Department of Surgery, "Ion Chiricuţă" Oncology Institute, Cluj-Napoca, Romania
[*]Authors with equal contribution

From an oncological perspective, the second most common malignancies in children are brain tumors. Despite the recent therapeutic breakthroughs in this field, concerning surgery, radiotherapy and chemotherapy alike, some cases still have poor outcomes in curability. This is especially the case in patients with high-risk histological types of tumors, and those suffering from residual, remitting and disseminated diseases. Due to the unique neuroanatomical emplacement of brain tumors and their aggressive infiltrative behavior, their total removal remains a demanding task. This can be perceived in the high rates of failure treatment and disease recurrence. Furthermore, the adjacent healthy brain tissue is inevitably damaged in the surgical process of effectively removing these tumors. Thus, stem cell transplantation may be a viable solution for the clinical management of these malignancies, as proven by various recent breakthroughs. In the current concise review, we present the role of next generation sequencing in HLA typing for stem cell transplantation in primary CNS pediatric malignancies.

Key words: Stem cell transplantation, pediatric CNS malignancies, HLS typing using sequencing.

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC ONCOLOGY

From an oncological perspective, the second most common malignancies in children are brain tumors [1, 2]. These also represent the most frequent form of solid tumors in this age group. Despite the recent therapeutic breakthroughs in this field concerning surgery, radiotherapy and chemotherapy alike, some cases still have poor outcomes in curability. This is especially the case in patients with high-risk histological types of tumors, and those suffering from residual, remitting and disseminated diseases [3, 4]. Over a decade, the therapeutic combination of high-dose chemotherapy and autologous hematopoietic stem cell transplant (AHSCT) has been evaluated in patients with high-risk tumors as a new method of eliminating residual malignant cells and improving curability [5].

Sandu and Schaller have stated that neural stem cells (NCS) have the remarkable ability to migrate toward pathologically affected areas in the central nervous system [6]. These pluripotent NCS traverse great distances and engraft within discrete areas, as well as diffuse neuronal abnormalities. This process may be followed by their incorporation into the local neuronal environment, with the ability of stable expressing of NSC genes. In this manner, the damaged neural tissues can be adequately replaced. Recent evidence has emerged, implying that successfully engrafted exogenous NSCs may promote neuroprotection and regeneration of the neural pathways of the host.

Due to the unique neuroanatomical emplacement of brain tumors and their aggressive infiltrative behavior, their total removal remains a demanding task. This can be perceived in the high rates of failure treatment and disease recurrence. Furthermore, the adjacent healthy brain tissue is inevitably damaged in the surgical process of effectively removing these tumors. Pathologically, brain malignancies are characterized by invasiveness, necrosis and angiogenesis, believed to be a result of chronic tissue hypoxia [7, 8]. A series of hypoxia-inducing factors are responsible for maintaining a balance between adaptation to hypoxia and apoptosis and/or necrosis in tumors, in turn facilitating metastases, recurrence, invasiveness and potential resistance to radiotherapy and chemotherapy. These factors are also believed to intercede the migration

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of NSCs to these particular locations [9].Owing to the inherent tropism of NCSs toward primary and invasive tumor foci, they can be utilized in supplying diseased tissues, including hypoxic tumor areas, with targeted therapeutic agents. A wide array of anti-tumor factors has already been successfully engineered into engraftable NSCs, including cytolytic viruses and genes coding for cytokines, prodrug-converting enzymes and neurotrophic factors. In animals with experimentally induced tumors, the transplantation of unaltered NSCs has extended survival, phenomenon that was further improved by the insertion of cytokine genes or pro-apoptotic genes in NSCs. However, these strategies require methodical *in vivo* monitoring. Nevertheless, there is reason to believe that transplantation of NCSs may actually aggravate tumor formation, since the existence of evidences suggesting that brain tumors may be caused by a single autologous NSC that failed to differentiate. A novel possibility would be the insertion of a failsafe suicide gene that would activate should transplanted cells fail to behave in a therapeutic manner [10-16].

The blood-brain barrier (BBB) is heavily altered within brain tumors. The vasculature is tortuous, with various degrees of structural integrity disruption, presence of arteriovenous shunts, irregular thickness and increased leakiness [17]. Normal BBB physiologically limits the delivery of therapeutic agents, including NSCs, therefore numerous surgical and pharmacological approaches have been obtained in circumventing it. Despite the common leakiness of the BBB in the core of the malignant tumors, the peripheral areas, which represent the actively proliferating regions of the tumors, are known to have inconstant and complex barrier integrity and permeability.

The advancement of molecular imaging has led to better understanding of the engrafted NSC kinetics, proliferation and viability *in vivo*. One such example is the noninvasive reporter gene assays extended into *in vivo* multimodality imaging platforms. These reporter genes can be introduced into cells using diverse vector and non-vector methods under the control of engineered promoters and enhancers. These genes can then be transcribed into bioactive proteins that are detected with sensitive noninvasive instrumentation by employing signal-generating probes (D-luciferin for optic imaging) [18]. The cells that have been stably transduced will deliver the reporter genes incorporated into the DNA to daughter cells. However, despite the possibility of longitudinal monitoring of NSC survival and proliferation *in vivo*, the clinical usefulness is restricted by poor tissue penetration and low spatial resolution and is therefore impractical for patient trials. Clinical magnetic resonance imaging (MRI) may be used to follow dynamic spatiotemporal patterns of NSC tumor targeting as this has high spatial resolution at 3 Tesla and remarkable soft tissue contrast. Research on MRI visualization of cellular tracking has known swift development especially in the past decade. Even so, with the higher specificity of positron emission tomography (PET) ligands and the ability of PET to detect reporter genes, it is likely that PET would also expand into clinical application for neurosciences. An ambitious method would be to engineer reporter genes directly into the NSC before engrafting and then to systematically inject the PET ligand to detect transporter cells, thus avoiding some of the negative long-term effects of contrast agents [19, 20].

There are a few presumably prognostic factors in patients harboring brain malignancies and treated with high-dose chemotherapy and hematopoietic stem cell transplantation. Such factors include the widely used N-myc amplification and 1p deletion, however other factors such as TRK, CD44 and MDR expression and ploidy are currently under investigation. The efficacy of different combinations employed to treat pediatric patients with neuroblastoma is known. One study also demonstrated a better prognosis in patients under 2 years of age, as well as the absence of bone-marrow metastasis at diagnosis and the presence of Busulfan-Melphalan combination in high-dose chemotherapy (HDCT) [21]. This paper also stated that the disease status of stage IV neuroblastoma did not appear to be a prognostic factor at the time of HDCT. Due to the fact that certain brain malignancies such as medulloblastoma have a tendency to relapse after surgical excision and subsequent irradiation, and the fact that they are sensible to chemotherapy, it has been suggested that chemotherapy could replace irradiation in preventing metastatic disease in locally relapsing young patients [22]. However, due to the high toxicity of HDCT with Busulfan and Thiotepa and autologous stem cell transplantation (ASCT) after surgery and irradiation in relapsing patients, this has proven as inefficient in improving the prognosis, despite the high response rate [23].

Secondino *et al*. [24] describe the case of a 25-year old woman with a previously radically treated and irradiated stage IV medulloblastoma who was reported to have developed chronic graft-*versus*-host disease (GVHD) after receiving hemato-

poietic stem cell (collected from her HLA identical brother) transplantation. Considering the significant impairment of the patient's quality of life, this was a major disappointment, despite the long-lasting remission that resulted from the otherwise expected graft-*versus*-tumor effect. The Italian experience is accompanied by another study from Japan, where Nishikawa *et al*. [25] presented the case of a 6-year old patient who underwent subtotal resection of a medulloblastoma, followed by four cycles of ICE (ifosfamide, cisplatin, and etoposide chemotherapy), whole craniospinal and local radiation therapy, and subsequent tandem high-dose chemotherapy (carboplatin, thiotepa and busulfan and melphalan) with autologous peripheral blood stem cell transplantation (PBSCT) died of busulfan-induced lung disease that caused respiratory failure. It was also reported that this patient had unexplained high busulfan areas under the drug plasma concentration-time curve (AUC) levels. This has led to the recommendation that all patients with high-dose busulfan levels should be monitored closely.

According to Lafay-Cousin [26], certain embryonic malignancies have undertaken maturation, which is an unusual phenomenon. This can occur either spontaneously, or following therapy, though its meaning is unclear. A 21-month-old girl who was diagnosed and subsequently treated for a supratentorial neuroectodermal tumor, presented with a recurrence after HDCT and stem cell transplantation. The recurrence turned out to have been less cellular more mature in histological appearance. Since the patient suffered a second recurrence that closely resembled the original tumor, it is uncertain whether the treatment played a decisive role in triggering this phenomenon. It is still unclear whether the maturation process serves as a prognostic factor, especially due to its rarity. An unusual case of recurrent metastatic medulloblastoma was reported in a 28-year-old man, presenting with an increasing cervical lymphadenopathy associated with the occipital scar of the neurosurgical treatment of the initial tumor [27]. The patient was treated with the standard Ewing sarcoma regimen (vincristine, doxorubicin, and cyclophosphamide (VDC) alternating with ifosfamide and etoposide (IE), considering that these agents are active in medulloblastoma. Subsequently, he followed HDCT with thiotepa, etoposide, and carboplatin with ASCT. After having finished the treatment with adjuvant radiation encompassing any involved site of recurrent disease, the report claims the patient is now still in complete remission.

Apheresis procedures prove to be challenging in pediatric patients, especially in children with low body mass [28], due to their low blood and erythrocyte volume levels. Therefore, to avoid hypovolemia and hypoxemia, the system is filled with blood. However, this lengthens the treatment time and decreases work efficiency. Vascular approach is another technical specificity in children.

It has also been described that malignant brain tumors in children treated with HDCT with busulfan-thiotepa and radiotherapy presented pseudoprogression [29]. Neurotoxicity was a delayed result and the radiological abnormalities in the irradiated field were transient, although early occurring. Most frequently, this phenomenon was associated with high-grade glioma, particularly with concomitant radiotherapy and temozolomide treatment. Pseudoprogression was described as temporary BBB alterations with neuroradiological features frequently indistinguishable from the progression of the disease. This is probably a good example of the clinical utility of diffusion-weighted imaging or functional PET scans, which have proven promising capabilities in differential diagnosis.

NEXT GENERATION SEQUENCING FOR TYPING

DNA sequencing was first used extensively in the Human Genome Project that took 13 years to be completed and was done using Sanger sequencing, which was developed by Sanger in 1975 and became the golden standard for DNA sequencing [30]. The need for cheaper and faster sequencing methods help the development of next generation sequencing technologies (NGS). Next Generation Sequencing (NGS) is a term that comprises different new sequencing technologies that were developed due to the need to overcome the limitation of the traditional Sanger sequencing. The newer sequencing technologies allow the user to sequence multiple samples shorter, cheaper and to obtain more data.

Since 2005 several NGS platforms were developed that use massive parallel sequencing to perform high-throughput sequencing. The most common used platforms now are Miseq, Hiseq and Nexseq from Ilumina, Ion Torrent Personal Genome Machine and Proton from Life Technologies, 454 GS Sequencers from Roche, Pacific Bioscience RS

and Solid from Applied Bioscience. Even though each NGS sequencer is unique they use similar protocols that are comprised of library preparation and quantification, template synthesis, sequencing and data analysis [31]. As an example the workflow for Ion Torrent PGM sequencer from Life Technologies is presented in Figure 1.

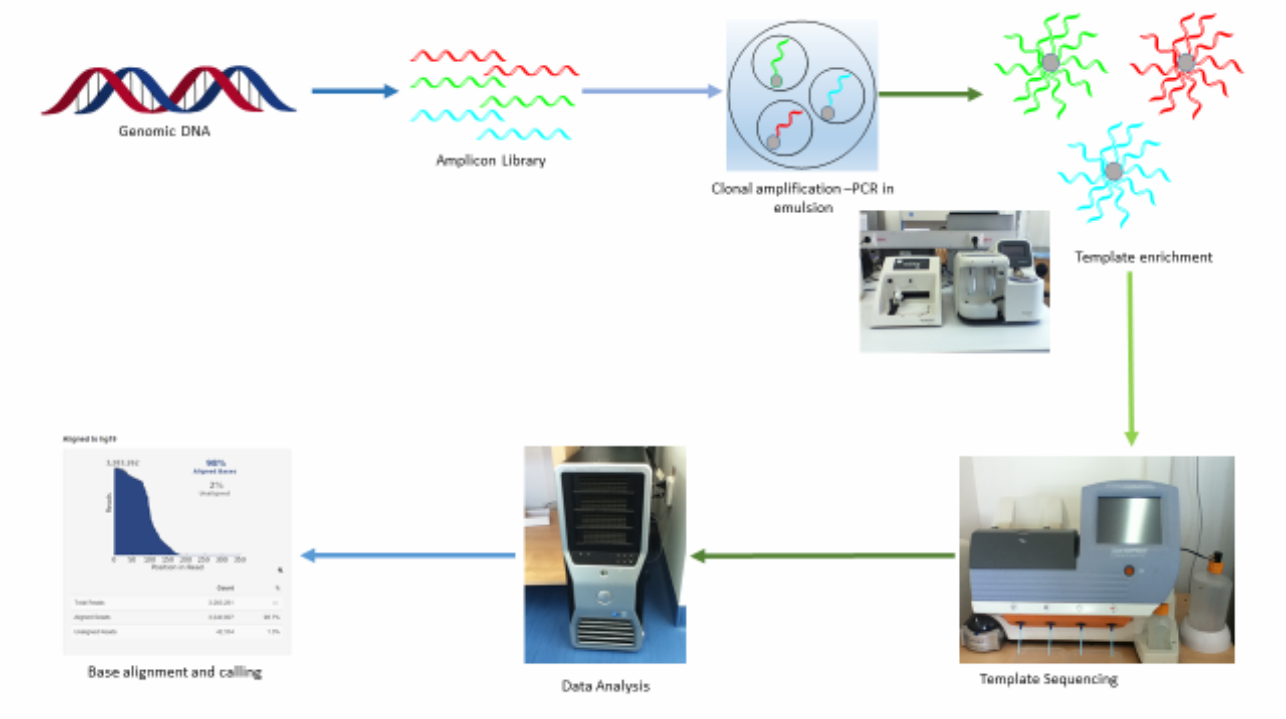

Figure 1. Ion Torrent PGM sequencing workflow.

As any technique also NGS has advantages and limitations. The main advantages are the lower cost, the shorter time, high-throughput and more application in comparison to Sanger sequencing. The limitations are given by the fact that even though NGS is cheaper than Sanger sequencing, still remains an expensive technique for some small laboratories. Also some mismatched in the homopolymer regions and the data analysis is time consuming and needs big storage space and bioinformatics knowledge, due to the high amount of data resulting from the sequencing experiments [32].

Due to its main advantages the NGS technology has gain high interest both in research and clinical diagnosis. The main applications of NGS are genome sequencing used in comparative biology and in public health field, RNA sequencing for the evaluation of the expression of RNA, target sequencing for evaluation of specific coding regions and epigenetic studies [33].

HLA GENOTYPING USING NGS

The HLA alleles described in the IMGT/HLA Database are over 10000[34]and most of the alleles differ from one another by a single base substitution. The difference in the HLA alleles is analyzed by several methods in different laboratories, the main methods employed for HLA typing are serological methods, mixed lymphocyte cultures and DNA-based methods. The DNA-based methods are composed of specific oligonucleotide probe hybridization (SSOP), sequenced specific primer amplification (SSP), sequencing-based typing (SBT) and reference strand-based conformation analysis (RSCA) [35]. In the recent years the “gold standard” for HLA genotyping has become Sanger sequencing, but due to the advantages of NGS, this technique has gained specific interest for HLA typing. Due to its advantages NGS gain a lot of interest for the HLA genotyping by sequencing and allele assignment.

Some of the market platforms that can be used for HLA genotyping are the MiSeq and HiSeq from Ilumina, 454 sequencers from Roche and Pacific Bioscience RS [31]. The technical sequencing specifications for these platforms are presented in Table 1.

The first NGS was used of HLA typing in 2009 by Gabriel C *et al*. [37] and Bentley G [38] both groups used the platform 454. Gabriel C *et al*. used the GS FLX 454 from Roche for HLA-A and B typing of 8 samples. Independently, Bentley G *et al*. were able to sequence using the same sequencer a 24 DNA sample from cell lines per run and 48 samples per run (24 DNA samples from cell lines and 24 DNA samples from blood) using the 454 protocol. For the 24 sample run they obtained a 99.4% concordance for all the 7 loci they sequenced.

Table 1
Technical sequencing specifications for some of the next generation sequencing platforms on the market [37, 38]

Technology	Input material	Run time	Report accuracy	Read Length	Output per Run	Raw Error
MiSeqIlumina	50-1000ng	4 h	mostly >Q30	up to 150bases	1.5-2Gb	0.8%
HiSeqIlumina	50-1000ng	11 days	mostly >Q30	up to 150bases	up to 600Gb	0.26%
Ion Torrent PGM	100-1000ng	2h	Mostly Q20	up to 150bases	20-50Mb on 314 chip, 100-200Mb on 316 chip, 1Gb on 318 chip	1.71%
PacBio RS	1000ng	2h	<Q10	average 1500 bases (C1 chemistry)	100Mb	12.86%

Several research groups have tried over the years to overcome the limitations of the NGS HLA genotyping, limitations given by small sample size, non-contiguous amplicons, multiple PCR for one loci, laborious library preparations or complex data analysis, by using different types of sequencers, library preparation methods or analysis methods.

In 2011 Holcomb *et al*. used the GS FLX 454 from Roche with the Conexio ATF software in an eight different laboratories study for the genotyping of the same 20 samples for 10 loci of the HLA genes and obtained an overall concordance of 97.2% [39]. Wang *et al*. used the IlluminaMiseq-sequencer for a high-throughput genotyping of 59 clinical samples for the four HLA loci (HLA-A, -B, -C and DRB1) in one run and obtained an accuracy of 99% [40]. In 2013 several groups published their results on HLA genotyping using the 454 sequencers from Roche. One study presented the genotyping of 173 samples in 18 GS Junior sequencer runs for 17 exons of the HLA-A, -B, -C, -DQB1, -DPB1, -DRB3, DRB4 and DRB5 with a read length of 400 bp and concordance of 97.3% analyzing 1242 loci from a total of 1273 loci [41]. In the other studies 192 samples were genotyped with a 100% concordance for the 8 loci studies and 96 samples per run. This study introduced a new approach for library preparation using the Fluidigm Access Array™ that helps simplifying the library preparation protocol [42].

The group of Chao presented in 2014 a study describing the usage of the BayesTyping 1 method for the assignment of three HLA loci using the simulated data for a PacBio circulating consensus sequencing read. They observed that even though this sequencer has the higher error rate by using the BayesTyping 1 method the PacBio sequencer limitations are overcome and one could identify the HLA alleles accurately [43]. Ehrenberg *et al*. used for HLA genotyping the IlluminaMiSeq sequencer using different sequencing protocols. One study used a multi-locus individual tagging method combined with NGS in order to evaluate 4 different HLA loci of 96 individuals in a single run. This method was able to call all HLA alleles and also to resolve ambiguities that could not be resolved by Sanger sequence-based typing (SBT) and at a cost similar to the SBT [44]. Smith *et al*. used the NGS for resequencing exon 2 and 3 of DRB1/B3/B4/B5, DQA1 and DQB1, and exon 2 of DPA1 and DPB1 of 2605 hematopoietic cell transplant recipients and donors and obtain 99.6% accuracy for DRB1 assignment and 99.5% for DQB1 compared to the alleles genotyped pre-transplant. They were also able to eliminate diploid ambiguities by in-phase sequencing [45]. In a study 79000 samples from China Marrow Donor Registry were genotyped for HLA –A, -B, -C, DRB1 and DQB1 loci. In this study 2068 samples were genotyped simultaneously in a HiSeq flow cell reducing the cost of the analysis by 95% compared to the SBT analysis and obtained 1100 new HLA alleles [46].

Taking into consideration all this study it is obvious that this technique has become a widely

used for HLA genotyping due to its high-throughput resolution, cost efficiency and high-accuracy, and it is possible that in the near future NGS will become the ”golden standard” for HLA genotyping.

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Din punct de vedere oncologic tumorile cerebrale sunt al doilea tip de cancer diagnosticat la copii. În ciuda descoperirilor recente în acest domeniu, în legătură cu operația, radioterapia sau chimioterapia, unele cazuri încă au un pronostic prost. Acest lucru se observă cel mai des la pacienții cu tumori cu risc crescut din punct de vedere histologic, sau în cazul pacienților care suferă de recurență sau diseminarea bolii. Datorită localizării neuroanatomice unice şi a comportamentului agresiv şi infiltrativ al tumorilor cerebrale, îndepărtarea lor totală este încă o sarcină dificilă. Ceea ce se poate observa în rata mare de eşec a tratamentului şi în numărul mare de recurențe ale bolii. Mai mult, țesutul cerebral sănătos adiacent este inevitabil deteriorat în timpul procedurii chirurgicale de îndepărtare a tumorii. Astfel, transplantul de celule stem poate să fie o soluție viabilă la managementul acestor tipuri de boli, după cum se poate vedea în cazul descoperirilor recente. În acest review concis, noi prezentăm rolul secvențierii de nouă generație în tipizarea HLA pentru transplantul cu celule stem în tumori maligne pediatrice ale SNC.

Corresppondence to: Ciprian Tomuleasa, MD, PhD, “Iuliu Haţieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania
Department of Hematology, “Ion Chiricuță” Oncology Institute, Cluj-Napoca, Romania
E-mail: ciprian.tomuleasa@umfcluj.ro

REFERENCES

1. RAVINDRANATH Y. *Evolution of modern treatment of childhood acute leukemia and cancer: adventures and battles in the 1970s and 1980s*. Pediatr Clin North Am. 2015; **62**(1):1-10.
2. BLEEKER FE., HOPMAN SM., MERKS JH., AALFS CM., HENNEKAM RC. *Brain tumors and syndromes in children*. Neuropediatrics. 2014; **45**(3):137-61.
3. MILLARD NE., DUNKEL IJ. *Advances in the management of central nervous system germ cell tumors*. Curr Oncol Rep. 2014; **16**(7):393.
4. THORP NJ., TAYLOR RE. *Management of central nervous system tumours in children*. Clin Oncol (R Coll Radiol). 2014; **26**(7):438-45.
5. CHEUK DK., LEE TL., CHIANG AK., HA SY., CHAN GC. *Autologous hematopoietic stem cell transplantation for high-risk brain tumors in children*. J Neurooncol. 2008; **86**(3):337-47.
6. SANDU N., SCHALLER B. *Stem cell transplantation in brain tumors: a new field for molecular imaging?* Mol Med. 2010; **16**(9-10):433-7.
7. COHEN AL., COLMAN H. *Glioma biology and molecular markers*. Cancer Treat Res. 2015; **163**:15-30.
8. WURDINGER T., DEUMELANDT K., VAN DER VLIET HJ., WESSELING P., DE GRUIJL TD. *Mechanisms of intimate and long-distance cross-talk between glioma and myeloid cells: how to break a vicious cycle*. Biochim Biophys Acta. 2014; **1846**(2):560-75.
9. NAKANO I. *Stem cell signature in glioblastoma: therapeutic development for a moving target*. J Neurosurg. 2015; **122**(2):324-30.
10. CHINTAGUMPALA M., GAJJAR A. *Brain tumors*. PediatrClin North Am. 2015 ;**62**(1):167-78.
11. THOMAS C., BHATIA S.*Cancer: brain-regulated biphasic stress response induces cell growth or cell death to adapt to psychological stressors*. Adv Mind Body Med. 2014; **28**(3):14-21.
12. AMBADY P., BETTEGOWDA C., HOLDHOFF M. *Emerging methods for disease monitoring in malignant gliomas*. CNS Oncol. 2013; **2**(6):511-22.
13. CARMIGNANI M., VOLPE AR., ALDEA M., SORITAU O., IRIMIE A., FLORIAN IS., *et al. Glioblastoma stem cells: a new target for metformin and arsenic trioxide*. J Biol Regul Homeost Agents. 2014; **28**(1):1-15.
14. ALDEA M., CRACIUN L., TOMULEASA C., BERINDAN-NEAGOE I., KACSO G., FLORIAN IS., *et al*. *Repositioning metformin in cancer: genetics, drug targets, and new ways of delivery*. Tumour Biol. 2014; **35**(6):5101-10.
15. ALDEA M., TOMULEASA C., PETRUSHEV B., SUSMAN S., KACSO GI., IRIMIE A., *et al*. *Antidiabetic pharmacology: a link between metabolic syndrome and neuro-oncology?* J BUON. 2011; **16**(3):409-13.

16. FLORIAN IS., TOMULEASA C., SORITAU O., TIMIS T., IOANI H., IRIMIE A., *et al. Cancer stem cells and malignant gliomas. From pathophysiology to targeted molecular therapy*. J BUON. 2011; **16**(1):16-23.
17. AIKEN R.*Molecular neuro-oncology and the challenge of the blood-brain barrier*. Semin Oncol. 2014; **41**(4):438-45.
18. SINGHAL T., ALAVI A., KIM CK.*Brain: positron emission tomography tracers beyond 18F fluorodeoxyglucose.* PET Clin. 2014; **9**(3):267-76.
19. JUHÁSZ C., DWIVEDI S., KAMSON DO., MICHELHAUGH SK., MITTAL S. *Comparison of amino acid positron emission tomographic radiotracers for molecular imaging of primary and metastatic brain tumors*. Mol Imaging. 2014; **13**.
20. WHITFIELD GA., KENNEDY SR., DJOUKHADAR IK., JACKSON A. *Imaging and target volume delineation in glioma.* Clin Oncol (R Coll Radiol). 2014; **26**(7):364-76.
21. HARTMANN O., VALTEAU-COUANET D., VASSAL G., LAPIERRE V., BRUGIERES L., DELGADO R, *et al*. *Prognostic factors in metastatic neuroblastoma in patients over 1 year of age treated with high-dose chemotherapy and stem cell transplantation: a multivariate analysis in 218 patients treated in a single institution*. Bone Marrow Transplant. 1999; **23**(8):789-95.
22. DUPUIS-GIROD S., HARTMANN O., BENHAMOU E., DOZ F., MECHINAUD F., BOUFFET E., *et al*. *Will high dose chemotherapy followed by autologous bone marrow transplantation supplant cranio-spinal irradiation in young children treated for medulloblastoma?*J Neurooncol. 1996; **27**(1):87-98.
23. VALTEAU-COUANET D., FILLIPINI B., BENHAMOU E., GRILL J., KALIFA C., COUANET D., *et al*. *High-dose busulfan and thiotepa followed by autologous stem cell transplantation (ASCT) in previously irradiated medulloblastoma patients: high toxicity and lack of efficacy*. Bone Marrow Transplant. 2005; **36**(11):939-45.
24. SECONDINO S., PEDRAZZOLI P., SCHIAVETTO I., Basilico V., BRAMERIO E., MASSIMINO M, *et al. Antitumor effect of allogeneic hematopoietic SCT in metastatic medulloblastoma*. Bone Marrow Transplant. 2008; **42**(2):131-3.
25. NISHIKAWA T., OKAMOTO Y., TANABE T., SHINKODA Y., KODAMA Y., HIGASHI M., *et al. Unexpectedly high AUC levels in a child who received intravenous busulfan before stem cell transplantation.* Bone Marrow Transplant. 2010; **45**(3):602-4.
26. LAFAY-COUSIN L., HADER W., WEI XC., NORDAL R., STROTHER D., HAWKINS C., *et al*. *Post-chemotherapy maturation in supratentorial primitive neuroectodermal tumors.* Brain Pathol. 2014; **24**(2):166-72.
27. CLEMENT J., VARLOTTO J., RYBKA W., FRAUENHOFFER E., DRABICK JJ. *Unusual case of recurrent extraneural metastatic medulloblastoma in a young adult: durable complete remission with Ewing sarcoma chemotherapy regimen and consolidation with autologous bone marrow transplantation and local radiation*. J Clin Oncol. 2013; **31**(19):e316-9.
28. VELJKOVIC D., VUJIC D., NONKOVIC OS., JEVTIC D., ZECEVIC Z, LAZIC E. *Mobilization and harvesting of peripheral blood stem cells in pediatric patients with solid tumors*. Ther Apher Dial. 2011; **15**(6):579-86.
29. NEGRETTI L., BLANCHARD P., COUANET D., KIEFFER V., GOMA G., HABRAND JL., *et al*. *Pseudoprogression after high-dose busulfan-thiotepa with autologous stem cell transplantation and radiation therapy in children with brain tumors: Impact on survival.* Neuro Oncol. 2012; **14**(11):1413-21.
30. SANGER F., NICKLEN S., COULSON AR. *DNA sequencing with chain-terminating inhibitors*. Proc Natl Acad Sci USA. 1977; **74**(12):5463-7.
31. QUAIL MA., SMITH M., COUPLAND P., OTTO TD., HARRIS SR., CONNOR TR., *et al. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers.* BMC Genomics. 2012; **13**:341.
32. METZKER ML. *Sequencing technologies – the next generation*. Nat Rev Genet. 2010; **11**(1):31-46.
33. GRADA A., WEINBRECHT K. *Next-generation sequencing: methodology and application*. J Invest Dermatol. 2013; **133** (8):e11.
34. MELDRUM C, DOYLE MA, TOTHILL RW. *Next-generation sequencing for cancer diagnostics: a practical perspective.* Clin Biochem Rev. 2011; **32**(4):177-95.
35. ROBINSON J., HALLIWELL JA., MCWILLIAM H., LOPEZ R., PARHAM P., MARSH SG. *The IMGT/HLA database*. Nucleic Acids Res. 2013; **41**(Database issue):D1222-7.
36. JUNG HL. *Shedding a new light on the HLA matching*. Korean J Hematol. 2011; **46**(1):1-2.
37. GABRIEL C., DANZEL M., HACKL C., KOPAL G., HUFNAGL P., HOFER K., *et al*. *Rapid high-throughput human leucocyte antigen typing by massive parallel pyrosequencing for high-resolution allele identification*. Hum. Immunol. 2009, **70**, 960-64.
38. BENTLEY G., HIGUCHI R., HOGLUND B., GOODRIDGE D., SAYER D., TRACHTENBERG EA, *et al*. *High-resolution, high-throughput HLA genotyping by next generation sequencing*. Tissue Antigens 2009, **74**, 393-403.
39. HOLCOMB CL., HÖGLUND B., ANDERSON MW., BLAKE LA., BÖHME I., EGHOLM M., *et al*. *A multi-site study using high-resolution HLA genotyping by next generation sequencing.*Tissue Antigens. 2011; **77**(3):206-17.
40. WANG C., KRISHNAKUMAR S., WILHELMY J., BABRZADEH F., STEPANYAN L., SU LF., *et al. High-throughput, high-fidelity HLA genotyping with deep sequencing*. Proc Natl Acad Sci USA. 2012; **109**(22):8676-81.
41. DANZER M., NIKLAS N., STABENTHEINER S., HOFER K., PRÖLL J., STÜCKLER C., *et al. Rapid, scalable and highly automated HLA genotyping using next-generation sequencing: a transition from research to diagnostics.* BMC Genomics. 2013; **14**:221.
42. MOONSAMY PV., WILLIAMS T., BONELLA P., HOLCOMB CL., HOGLUND BN., HILLMAN G., *et al*. *High Throughput HLA genotyping using 454 sequencing and the FluidigmAccess Array™ system for simplified amplicon library preparation*. Tissue Antigens 2013, **81**, 141-49.
43. CHANG CJ., CHEN PL., YANG WS., CHAO KM. *A fault-tolerant method for HLA typing with PacBio data*. BMC Bio-informatics. 2014; **15**:296.
44. EHRENBERG PK., GERETZ A., BALDWIN KM., APPS R., POLONIS VR., ROBB ML., *et al. High-throughput multiplex HLA genotyping by next-generation sequencing using multi-locus individual tagging*. BMC Genomics. 2014; **15**:864.
45. SMITH AG., PYO CW., NELSON W., GOW E., WANG R., SHEN S., *et al. Next generation sequencing to determine HLA class II genotypes in a cohort of hematopoietic cell transplant patients and donors*. Hum Immunol. 2014; **75**(10):1040-6.
46. ZHOU M., GAO D., CHAI X., LIU J., LAN Z., LIU Q., *et al*. *Application of high-throughput, high-resolution and cost-effective next generation sequencing-based large-scale HLA typing in donor registry.* Tissue Antigens. 2015; **85**(1):20-8.

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