Translational Medicine Communications

RESEARCH Open Access

Precision medicine in relapsed or refractory pediatric solid tumors: a collaborative Spanish initiative

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Abstract

Background: Understanding pediatric cancer biology is a huge challenge in continuous development that is currently being implemented into the clinical practice thanks to the new high throughput technologies integrated by personalized medicine.
We present the results of the Precision Medicine program for children and adolescents with solid tumors in relapse/progression carried out in University La Fe Hospital (Valencia) from 2014. This is the first Spanish experience in precision medicine published in pediatric oncology.

Methods: Study enrollment was offered to all patients having a refractory or relapsed solid tumor and an available biopsy treated in La Fe Hospital (Valencia, Spain) or in other Spanish pediatric oncologic center. Eighty four patients were finally studied. The commercial *Human Comprehensive Cancer GeneReadDNAseq Targeted genes Panel* (Qiagen©) was sequenced in fresh/frozen samples. Variants considered pathogenic or likely pathogenic were classified using the algorithm published by Parsons et al. based on perceived clinical utility.

Results: Thirteen of 84 patients (15%) received therapeutic recommendations due to an *actionable variant* detected and three patients received prognosis information based on sequencing results.

Conclusions: Precision medicine projects based on targetable gene panel approximations can obtain translatable information to pediatric patients with reasonable efforts. This approach lowers economic expenses and reduces time of response with respect to whole exome sequencing. Since the translation to the clinical practice is the main objective of these projects, limiting the number of relatively well-known biological markers will allow us to transfer similar amount of information with less economic and human effort.

Keywords: Precision medicine, Relapsed/refractory solid tumors, Genomics, Actionable pathways, Target therapy, Clinical translation

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Background

Cure rates for childhood cancer have progressively improved in the last decades and are impressive relative to those for adult's malignancies, reaching 75–80% 5-year survival rates [1]. However, cancer remains the leading cause of death by disease among children over 1 year of age in developed countries. Despite global good results, survival is still low for children with poor prognosis cancers such as high-grade glioma, brain stem tumour and metastatic medulloblastoma, sarcoma and neuroblastoma. Furthermore, progression or relapse for most pediatric patients with cancer still correlates with poor survival independently of different treatment combinations.

The exponential growth of next generation sequencing technologies (NGS) has allowed a generalized application [1, 2]. This has driven a rapid increase in molecular knowledge of tumors (especially in adults) [3], and as a result, the development of targeted treatments [4]. The new molecular targeted therapies have achieved unthinkable success in medical oncology, not so many years ago [5–8]. A significant percentage of adult patients may beneficiate from them after molecular tumor study [9]. Incidence and spectrum of cancers occurring in the pediatric population is markedly different from that seen in adults. Pediatric cancers have different type and frequency of genomic alterations compared to adult malignancies [10]. However, as recently demonstrated by Mody et al. [11], it is feasible to integrate sequencing data into clinical management of childhood cancers, aiming to characterize the landscape of genomic alterations in pediatric cancers, an essential step to improve outcome.

The need to explore the usefulness of drugs developed for adult patients arose among pediatric oncologists. The limited number of clinical trials available in children, as well as the lower biological knowledge of pediatric tumors led to the development of important precision medicine projects around the world [11–17]. In addition, the urgency to transfer biological information to patients in relapse/progression forced to start independent projects.

We present the results of the Precision Medicine program for children and adolescents with solid tumors in relapse/progression carried out in La Fe University Hospital (Valencia) from 2014. As far as we know this is the first published Spanish experience in precision medicine in pediatric oncology.

Methods

Study subjects and data collection

Study enrollment was offered to all patients having a refractory or relapsed solid tumor and an available biopsy treated in La Fe Hospital (Valencia, Spain) or in other Spanish pediatric oncologic center. High risk tumors were exceptionally considered and studied during first line treatment based on a probability of 5-year survival less than 20%. No other inclusion or exclusion criteria were required. The implementation and ultimate responsibility fall upon La Fe Hospital, but also several centers sent patients after approving the study protocol in the corresponding ethics committee. The program was approved by the Ethics committee of Hospital La Fe and the Pediatric Molecular Tumor Board (PMTB) was created in November 2014, composed by pediatric oncology, genetics, pharmacology, pathology, bioinformatics and molecular biology specialists. Parents signed the informed consent and were informed about the possibility of finding germ line mutations and accepting or refusing to be informed about that. When genomic and histopathological studies were completed, all members discussed the results and finally a report was transferred to the corresponding medical doctor. The workflow was based on the published INFORM pilot study [15]. The outcome of patients, including the treatment received, was updated on October 2018, through request to the responsible doctors.

Study samples

Fresh tumor samples from last relapse were requested. Paraffined and/or pretreatment tumor samples were used only if fresh samples were not possible to obtain. Peripheral blood samples were simultaneously collected in all cases. All tumour samples were reviewed by a board-certified pathologist to confirm histology and estimate tumour content. Only samples with > 30% tumour cell content were submitted for further genomic testing. The Pathologist selected the most cellular region from tumor samples and performed immunochemistry and FISH techniques when requested. The selected tumor material and peripheral blood samples were sent to the Biobank for DNA extraction and the Genomics Unit for sequencing analysis.

DNA extraction

DNA extraction from tumor samples was carried out using the *QIAamp® DNA Investigator kit* (QIAGEN® ref.56504); the DNA obtained was dissolved in TE Low EDTA buffer (AFFIMETRIX® ref. 75,793). Because the *QIAampminElute* (QIAGEN®) columns co-purify both RNA and DNA, 5 μL of a solution of RNase A (10 mg) was added/mL (Mat No. R1455S MN®), since RNA-free DNA is needed.Blood samples were collected in Vacutainer® tube with anticoagulant K2EDTA 5.4 mg of adequate volume for the purpose (2 ml). DNA extraction was carried out using the commercial kit *QIAamp® DNA Mini Kit* (QIAGEN® ref. 51, 304); the DNA obtained was dissolved in TE Low EDTA buffer (AFFIMETRIX® ref. 75,793).

DNA samples were preserved at the end of the extraction and up to the time of collection, at − 20 °C. The concentration by spectrophotometry, and absorbance ratios were measured with the NANODROP 2000®.

Sequencing studies, data interpretation and variant calling

DNA from tumor sample was studied using Ion Torrent technology (average coverage with a minimum of 1000X and homogeneity with a minimum of 85%).

Two commercial panels were used, the *Ion Ampliseq Cancer Hotspot Panel v2* was applied in paraffin samples and the *Human Comprehensive Cancer GeneReadDNAseq Targeted genes Panel* (Qiagen©) in fresh/frozen samples. This second one allows studying through sequencing technology (NGS) the coding regions (exons) and flanking intronic regions of the 159 genes most frequently mutated in cancer (Additional file 1: Table S1). *Ion Ampliseq Cancer HotSpots v2* (Thermo Fisher Scientific©) was sequenced using Ion Torrent technology (average coverage with a minimum of 1000X and uniformity with a minimum of 90%) (Additional file 2: Table S2).

Target validation with IHC/FISH and copy number assessment were undergone when needed, in order to enrich panel information.

Copy number assessment and LOH were detected by high resolution molecular karyotyping genomic array (Affymetrix CytoScan HD) in 250 ng DNA for fresh tissue and 1 μg for Formalin-Fixed Paraffin Embedded (FFPE), following manufacturer directions. Integrity was above 500 nucleotides. Threshold filters for gains and losses were set up to 100–500 kilobases (KB) depending on quality of sample (FFPE or fresh frozen tumour).

For the analysis of the panel results, a screening of genetic variants detected both in blood and tumor was firstly carried out; those selected variants were categorized as "germline variants". Secondly, variants detected exclusively in tumour were categorized as "somatic variants". In both cases, variants described as pathogenic or probably pathogenic in the literature or in diseases databases (ClinVar, HGMD, St Jude PeCan or CiVIC), with an MAF < 0.01, were always selected. For the rest of variants, an algorithm of filters have been applied to discard those with an allelic frequency < 5%, changes in non-coding regions (excluding splicing sites flanking the exon to +/− 10 nucleotides), synonymous variants (excluding splicing sites in +/− 4 positions), variants with high frequency in the general population (MAF > 0.01) or in our own database of Genomics Unit La Fe and polymorphic changes (SNPs) without clinical relevance found in healthy population or described as benign by several sources.

Variant calling was based in international recommendations as Pathogenic, Likely Pathogenic, Benign, Likely Benign, and Uncertain Significance [18]. In all cases the annotation of variants was based on the genome version GRCh37 (hg19). Variants considered pathogenic or likely pathogenic in multidisciplinary committee were confirmed using Sanger technique.

In order to more complete interpretation of genetic variants from previous bibliography and considering the lack of standards around the term "actionable findings or clinically relevant", we classified variants using the algorithm published by Parsons et al. [14]. They developed a ranking of variants based on perceived clinical utility and established 4 categories: established clinical utility (I); potential clinical utility (II); mutations in consensus cancer genes (III); and all other mutations (IV). The significance that they assigned to every group was [14]:

-Group I: Mutations known to be diagnostic, prognostic, and/or predictive of treatment in the specific tumor type tested.
-Group II: Mutations in members of targetable cancer pathways, gene families, or functional groups, regardless of tumor type.
-Group III: Mutations in other consensus cancer genes, not currently considered targetable.
-Group IV: All other mutations.

The term *actionable variant* causes important controversies depending on the specific tumor type and clinical-biological characteristics of patients. In our work, an *actionable variant* is referred as a genomic change that suggests an alteration with biological activity that could be targeted with a concrete therapy already used in vivo. Targeted therapies were preferentially recommended to be administered within clinical trials but also as compassionate use when this was not possible.

Other biologic studies

Based on previous bibliography and according to the sequencing results obtained for each tumor type, SNP CGH array, immunochemistry techniques (p-AKT, PDL1, p-EGFR, c-KIT, PTEN, Her2neu, p53) and/or FISH (NTRK1 / 3, ALK, BRAF) were also performed if required by the study doctor and the PMTB.

The number of pathogenic or likely pathogenic variants that were detected using *Comprehensive* or *HotSpot* panels and classified as categories I, II or III (Parsons et al. [14]) were contrasted. We have done a description of our results and also we have compared them with Parsons and other published data.

Results

These results are referred to sequencing results. Histopathological contributions and SNP array information are not communicated.

Patient and sample characteristics

Peripheral blood and tumor samples of 98 patients with solid tumors, from 19 Spanish pediatric oncology units (POU), were remitted to our program from November 2014 to October 2018 (48 months). Finally, 14 patients were not studied because of poor quality of tumor samples. Therefore, tumor and peripheral blood samples from 84 patients were analyzed. The most represented tumor was neuroblastoma with 29.8% of cases (25/84). The rest of the cohort was composed by nine ewing Sarcomas (10.7%), seven rhabdomyosarcomas (8.3%), six osteosarcoma samples (7.1%), three high grade glioma (3.5%) and others (34/84, 40.5%).

Seventy one patients were in relapse or progression, 48 in first and 24 in second or higher relapse or progression (second relapse/progression 12, third 8, fourth 4 and one patient in fifth relapse/progression). Another 12 patients were studied at debut (because high risk disease; 12/84, 14%) and one refractory patient completed the cohort. Tumor samples studied were obtained at relapse in 55 cases and the remaining 29 at tumor diagnosis. The median patient age was 9,9 years; 54% of patients were male and 46% female (Table 1).

Pathological diagnosis was modified after pathologist review in two cases (a patient from embryonal to alveolar rhabdomyosarcoma based on the PAX3-FOXO1A translocation and a patient from neuroblastoma to undifferentiated sarcoma). Median time between biopsy/surgery and molecular tumour board recommendation was 25 days (12–80 days).

Tumor sequencing

Fifty Tumor samples (60%) were studied through *Comprehensive* gene panel and 34 (40%) with *HotSpot* gene panel (Table 1). Variants classified as pathogenic or likely pathogenic using international system classification [18] were also distributed in the four Parsons categories. We detected a total number of 38 pathogenic or likely pathogenic variants in the overall patients (Table 2). A number of six patients (6/84, 7%) carried on pathogenic or likely pathogenic somatic variants with established clinical utility (category I): four Neuroblastoma patients with *ALK* mutations and two Aggressive Fibromatoses patients with *CTNNB1* mutations (Table 2). Pathogenic or likely pathogenic variants in potential utility genes (category II) were detected in eight patients (8/84, 9, 5%) (Table 2). Nineteen patients (19/84, 22,6%) carried variants in consensus cancer genes (category III) (Table 2). Therefore, 31 of 84 patients (36,9%) carried at least one variant considered as category I, II or III. Genetic variants classified at these categories may be directly actionable, considered as prognosis markers in specific tumor histologies and/or considered as biologically informative. Thus, these variants could be useful when taking therapeutic decisions. Two patients carried variants included in category IV of Parsons et al. classification (variants in *TERT* and *TERT* plus *ERBB3* in two Neuroblastoma patients) (Additional file 3).

The number of variants (categories I, II and III) detected in tumor samples by *Comprehensive gene panel* was higher than those detected by *HotSpot* gene panel. Indeed, we detected at least one variant classified as category I, II or III in 11 patients (11/34; 32.4%) using *HotSpot* gene panel. However, *Comprehensive* panel picked up at least one of these variants (category I, II or III) in 24 patient samples (24/50; 48%).

Germline sequencing

The germ line's approach was completed only in those patients studied through the *Comprehensive panel*, since February 2016. *Comprehensive panel* includes 90 predisposing pediatric cancer genes, based on Zhang et al. published data [19]. Then, 40 patients were studied. Globally, genetic changes certainly responsible of tumor development were detected in 4 cases (4/40; 10%). Specifically, 3 Li-Fraumeni patients were detected, two de novo patients (High Grade Glioma: *TP53* p.Arg273His and Choroid plexus carcinoma: *TP53* p.Arg273Cys) and one member of a Li-Fraumeni family no previously diagnosed (malignant meningioma: *TP53* p.144Ter). (Table 3). Their families were studied in Genetic Counseling Unit. Moreover, one *PTEN* hamartoma syndrome was diagnosed. Other pathogenic or likely pathogenic germline variants were detected in three patients, but the relationship with tumor predisposition was doubtable and therefore, this information was not translated to patient or family (Table 3).

Clinical translation

When variants were discussed in the PMTB, a report was prepared. Thirteen patients received therapeutic recommendations due to an *actionable variant* detected and three patients received prognosis information based on these sequencing results. Clinical recommendations from the PMTB according to the results from molecular studies at different tumor types are presented in Table 4. Clinical evolution in treated patients is summarized in Table 4.

Discussion

Advances in molecular biology and targeted therapies development have led to the evolution of precision medicine (also named personalized medicine) thanks to decreases in cost and time of sequencing technologies. The principle of precision medicine in this new era consists on identifying genomic actionable alterations in the patient's tumour. Over 20% of children with cancer will die from disease, either by relapse or non-response to standard treatment. Hence, improving knowledge on

Table 1 Patient characteristics

Characteristic	No (n=84)
Diagnosis	
Neuroblastoma	25
Ewing Sarcoma	9
Rhabdomyosarcoma	7
Osteosarcoma	6
High Grade Glioma	3
Ependymoma	3
Pilocytic Astrocytoma	3
Wilms tumor	2
Agressive Fibromatosis	2
Pilomyxoid Astrocytoma	2
Infantile fibrosarcoma	2
Other tumors (1 sample)	20
Age (years)	
0-4	19
5-8	18
9-12	22
13-17	20
18 or >18	5
Sex	
Male	45
Female	39
Clinical status (at enrollment)	
Debut (high risk tumor)	12
Relapse/progression	71
Relapse ≥ 2	25
Refractory	1
Tumor sample submitted	
Debut	29
Relapsed tumor	55
Panel sequenced	
Comprehensive	50
Hot Spot	34

Table 2 Tumor sequencing results; Pathogenic or likely pathogenic variants distributed in Parsons et al. categories

Catheghory	Gene	variant	Tumor type	Panel performed
I	*CTNNB1*	p.S45P	Aggressive Fibromatoses	*Hot Spot*
I	*CTNNB1*	p.S45P	Aggressive Fibromatoses	*Comprehensive*
I	*ALK*	p.R1275L	Neuroblastoma	*Comprehensive*
I	*ALK*	p.R1275Q	Neuroblastoma	*Comprehensive*
I	*ALK*	p.F1245C	Neuroblastoma	*Comprehensive*
I	*ALK*	p.R1275Q	Neuroblastoma	*Comprehensive*
II	*PTEN*	PTEN c.635-A>G	Hamartoma	*Hot Spot*
II	*BRAF*	p. V600E	Anaplastic oligodendroglioma	*Comprehensive*
II	*BRAF*	p. V600E	Pilocytic astrocytoma	*Hot Spot*
II	*BRAF*	p. V600E	Pilocytic astrocytoma	*Hot Spot*
II	*PI3KCA*	p.Glu542Lys	Rhabdomyosarcoma	*Hot Spot*
II	*KIT*	p. Pro577Ser	Neuroblastoma	*Hot Spot*
II	*MET*	p. Thr1010Ile	Neuroblastoma	*Hot Spot*
II	*EGFR*	p.His1111Tyr	Neuroblastoma	*Comprehensive*
III	*JAK3*	p. Pro996Thr	Choroid plexus carcinoma	*Comprehensive*
III	*DICER1*	P. Tyr1180Ter	Pineoblastoma	*Comprehensive*
III	*ATM*	p.Trp2344Ter	Malignant peripheral nerve sheath tumors	*Comprehensive*
III	*ATM*	p.Arg3008His	Neuroblastoma	*Comprehensive*
III	*TP53*	p. Cys135Phe	Ewing sarcoma	*Hot Spot*
III	*TP53*	p.Arg248Trp	Ewing sarcoma	*Hot Spot*
III	*TP53*	p.Arg273Cys	Rhabdomyosarcoma	*Hot Spot*
III	*TP53*	c.672+1G>C	High grade glioma	*Comprehensive*
III	*TP53*	p.Gly245Ser	High grade glioma	*Comprehensive*
III	*TP53*	p.Arg273His	High grade glioma	*Hot Spot*
III	*TP53*	p.Pro152Leu	Neuroblastoma	*Comprehensive*
III	*Rb1*	p. Gln121Ter	Osteosarcoma	*Comprehensive*
III	*KMT2D*	p.His2071dup	Rhabdomyosarcoma	*Comprehensive*
III	*NF1*	c.4577+1G>T	Neuroblastoma	*Comprehensive*
III	*ATRX*	p.Gly1965Ser	Neuroblastoma	*Comprehensive*
III	*ATRX*	p.Arg1743Ser	Neuroblastoma	*Comprehensive*
III	*ARID1*	p.Gly1298Ter	Neuroblastoma	*Comprehensive*
III	*ARID1*	p.Gln2212Ter	Neuroblastoma	*Comprehensive*
III	*SMARCB1*	Trp131Val	Neuroblastoma	*Comprehensive*
III	*TSC2*	p.Lys1585Pro1589delinsThr	Neuroblastoma	*Comprehensive*
III	*TERT*	p.Ser586Asn	Neuroblastoma	*Comprehensive*

Table 3 Germline results (*n*=40); pathogenic or likely pathogenic variants detected

Tumor type	Variant detected	Tumor-variant relation
Hamartoma	*PTEN* c.635-A>G	Predispose
Choroid plexus carcinoma	*TP53* p.Arg273Cys	Predispose
Malignant meningioma	*TP53* p.144Ter	Predispose
High grade glioma	*TP53* p.Arg273His	Predispose
Ependymoma	*ATM* p.R982C	unknow
Neuroblastoma	*SMARCA4* p.Gly233fs*62	unknow
Neuroblastoma	*FANCA* p.D79Vfs*15 *ERCC4* c.584+1G>A	unknow

their tumour biological profile at relapse/progression may identify potential actionable alterations for which molecularly matched treatments are available and poor prognosis patients could benefit from [20]. In this context, different personalized medicine projects have been developed around the world and common difficulties discovered are well summarized by Moody et al. [21].

The first challenge in precision medicine is to decide how to look for potential targetable markers. Few biomarker-driven treatments are in clinical use in pediatric oncology currently, such as specific BRAF inhibition in *BRAF* V600E pediatric gliomas [14, 15] and ALK kinase inhibitors in *ALK* translocated anaplastic large cell lymphomas [22], ALK translocated inflammatory myofibroblastic tumours and ALK mutated neuroblastomas [16]. Thus, ALK and BRAF testing in selected tumours at relapse/progression is a standard of care in most pediatric oncology centres. However, consecutive single-marker testing is not compatible with clinical practice because of limited amount of tumour available (mostly diagnostic tru-cut), the intrinsic delays in each analysis and the overall cost [17]. The rapid development of high-throughput technologies and computational frameworks enables the examination of biological systems in unprecedented detail. It allows identify less common but targetable alterations across diseases. Nowadays, many pediatric institutions are starting to build molecular screening programs in order to treat patients according to their genomic alterations. The extent of genomic studies ranges from commercial hotspot cancer panels developed for adult tumours to whole exome sequencing (WES), RNA sequencing and methylation arrays [5, 19]. Noteworthy, the exome represents approximately 1% of the genome and harbour about 85% of the disease driven mutations. However, the larger analysis difficult its interpretation and a comparison with normal tissue is recommended to separate somatic from constitutional variants. After evaluating the different ways to face this first challenge we opted to use a panel of genes.

Pediatric cancer present a low mutation rate comparing with the adults [23, 24], however, the availability of specific drugs for the identified genetic alterations found is still limited, making difficult the approximation of precision medicine to pediatric tumors [25–27]. Only few genetic alterations are really actionable in concrete tumors and in small patient cohorts [28], besides that the availability of targeted drugs for children with cancer is scarce. Therefore, deeper tumor knowledge and specialized drugs are necessary. However, target drugs developed in adulthood tumors can be useful in some pediatric patients, justifying the huge effort invested in

Table 4 Clinical translation

Tumor histology	Actionable Variant detected	Other useful biologic information	Committe Recommenda-ttion	Treatment administred (Yes/No)	Time treated and response observed	Treatment abandoned (Yes/No)	Cause of abandon
Hamartoma	*PTEN* c.635-A>G	No	mTor inhibitor	Yes (*Everolimus*)	4 years 3 months (Partial response)	No (ongoing)	-
Neuroblastoma	*ALK* p.R1275L	No	ALK inhibitor	Yes (clinical trial; LDK378)	2 months (disease progression)	Yes	Progression
Neuroblastoma	*ALK* p.R1275Q	No	ALK inhibitor	No (patient/family decision)	-	-	-
Neuroblastoma	*ARID1* p.Gly1298Ter	No	mTor inhibitor and bad prognosis factor	No (patient dead)	-	-	-
Neuroblastoma	*ALK* p.F1245C	No	ALK inhibitor	Yes (crizotinib)	0 months (not tolerated)	Yes	Patient rejection
Neuroblastoma	*ALK* p.R1275Q	No	ALK inhibitor	No (future option)	-	-	-
Neuroblastoma	*TSC2* p.Lys1585Pro 1589delinsThr	No	mTor inhibitor	Yes	Not available	Yes	Not available
Rhabdomyosarcoma	*PIK3CA* p.Glu542Lys	P-AKT inmunochemistry positive in 40% tumor cells	mTor inhibitor (nor other drugs available)	No (future option)	-	-	-
Pylocitic astrocytoma	*BRAF* V600E	no	BRAF inhibitor	No (future option)	-	-	-
Pylocitic astrocytoma	*BRAF* V600E	no	BRAF inhibitor	No (future option)	-	-	-
Malignant peripheral nerve sheath tumors	*ATM* p.Trp2344Ter (homozigous)	no	Temozolamide plus PARP inhibitor	Yes (TMZ + Olaparib)	2 months (disease progression)	Yes	progression
Neuroblastoma	*TP53* p.Pro152Leu *TERT* p.Ser586Asn *ATRX* p.Arg1743Ser	no	Radiotherapy and/or topoisomerase II inhibitors And bad prognosis	Yes	5 months (partial response)	No (ongoing)	-
Neuroblastoma	*ARID1* p.Gln2212Ter	1p deletion	EZH2 inhibitor (Tazemetostat) and bad prognosis	No (future option)	-	-	-
Rhabdoid tumor	none	PDL1 inmunochemistry positive in 85% tumor cells	Check point (PD1/PDL1) inhibitor	No (patient dead)	-	-	-
Epithelioid PEComa of Kidney	none	P-AKT inmunochemistry positive in 100% tumor cells	mTor inhibitor	Yes (Sirólimus + Sorafenib)	5 months (stable disease)	Yes	progression
Osteosarcoma	none	mTor inmunochemistry positive in 60% tumor cells	mTor inhibitor	Yes (Sirolimus monotherapy; patient was clinically advanced)	2 months (disease progression)	Yes	progression

transferring this knowledge into pediatric oncology. This is why our group also joined the Personalized Medicine initiative in pediatric oncology. The identification of potentially actionable genetic variants in relapsed pediatric cancers varies from 30 to 60% between studies [21]. Unfortunately, patients receiving targeted therapy is even lower (3–18% of study populations) [29] and our study obtains similar rates as previously described results. The sequencing method performed is an important point to consider regarding the rate of identified genetic variants. For instance, we observed different percentages of genetic variants classified as category I, II or III, depending on the sequencing panel used (32% of patients had at least one mutation by *HotSpot* vs 48% of patients by *Comprehensive* panel). In our results, this difference is mainly due to *ALK* gene mutations that are not completely covered in *HotSpot* panel. We also compared our results with Parsons et al. conclusions. They used whole exome sequencing (WES) to study 121 patients'samples. They found four patients with category I genetic variants (3%), 29 patients with category II genetic variants (24%) and 24 patients with at least one category III genetic variant (20%). Therefore, 47% (57/121) of their patients had at least one genetic variant classified as either grade I, II or III. Although statistical comparisons with our results is not possible because the sequencing method and cancer population were different, it is interesting to remark that most of variants classified as category I, II and III in Parsons study were located in genes included in *Comprehensive* panel (Table 5). PEDS-MIONCO SEQ, Basic3, PIPseq, MAPPYACTS and INFORM Precision Medicine programs in pediatric oncology also use WES and RNA-seq and in some of them metilation and pharmacogenetic studies. Their results have been of

Table 5 Genes not included in *Comprehensive* panel where at least one variant (cathegory I, II or III) was detected in Parsons et al. study

ABL2	MN1	SET
ARID1B	NDRG1	TET2
CANT1	NONO	TNFRSF17
CDX2	NOTCH3	TPR
DDX3X	NSD1	
FGFR1	NTRK2	
FOXO3	PRCC	
FZD6	RMB15	
MAP2K7	RNF213	

outstanding importance to discover the genetic alterations of the pediatric tumors, and have permitted that other centers with more limited technical and/or economical resources could implement programs of Precision Medicine for their patients. Therefore, many genes studied in these projects have never been described mutated in pediatric cancer and consequently the clinical utility of WES or RNAseq compared to gene panels is inconclusive [30, 31]. Our results support the clinical utility of gene panels in pediatric oncology. However, copy number variations (CNV) have been considered an important aspect in pediatric tumors and we were not able to study it in many of our patients. This is considered a program weakness. Indeed, we have designed a specific targeted gene panel that encloses also CNV and some frequent translocations in pediatric cancer in order to maximize the coverage of genetic variants in the pediatric cancer population, maintaining an affordable budget for personalized clinical care.

The second challenge is how interpret detected variants. Based on this work, we want to underline the importance of the multidisciplinary PMTB in order to interpret genetic variations and give clinical recommendations. In our case PMTB was able to provide therapeutic interpretations of molecular profiles for the majority of patients, and targeted therapies were recommended for 13 of the cases. PMTBs ability to offer clinically useful interpretations of current molecular profiles required (i) the synthesis of published evidence about the prevalence of observed alleles and their documented pathogenicity, (ii) inference of potential pathogenicity based on molecular and signaling pathway modeling, and (iii) inference of potential therapeutic susceptibility based on the apparent allelic frequencies of observed mutations and known drug mechanisms of action. In precision medicine, additional questions can contribute to the challenge when a therapeutic decision must be taken by a MTB: *which role has a specific genetic alteration in the biology of the tumor?*; *what response rate to a targeted therapy is possible in a tumor type and in a concrete patient?*; *what bypass mechanism is present in the tumor when progressing again during target therapy?*. In order to clarify these issues, biomarker standardization efforts have been started [32, 33]. Although the exact utility of targeted drugs is still in growing knowledge, umbrella or basket trials derived from personalized medicine projects are necessary [29]. Although taking decision in an MTB from preclinical or clinical previous limited information in pediatric patient results very difficult is the best way to take conclusions.

Another challenge in precision medicine is also how to proceed with germ line variants. Significant family information is derived from personalized medicine studies and in some cases that is discovered without clinical suspicion. Therefore, both patients and parents must be properly informed about the possibility of being carriers of a germline variant, consequences or therapeutic possibilities in that case, with previous signed consent. In our work, we carried out family segregation studies in 4 patients with germline variants known as cancer predisposition variants.

The last challenge in middle size centers is the lack of investment in precision medicine platforms and in bioinformatic expert's support. Nevertheless, our efforts could get important information to our patients. We have compared both, our results and Parsons' in order

to decide the best way to continue. Although more translatable information might be obtained from studies of whole exome sequencing, RNA sequencing or epigenomics, these broad studies are not economically achievable in our work context. We must decide if the best option is working with panels no bigger than few hundred genes and design specific strategies for every tumor. An alternative strategy could be sequencing a small number of genes, analyzing the expression of certain proteins and performing analysis of copy number variations (CNV) or epigenomics on concrete genes (based on preclinical and published clinical data), according to each tumor type. Therefore, we have designed a directed gene panel, which includes all known predisposing genes to pediatric cancer [19] and actionable genes (direct or indirectly) in pediatric malignant solid tumors. This panel will enable the analysis of CNV as well as specific targetable genomic translocations. Finally, for the best of our knowledge, it is obvious that all required biologic data from clinical trials must be tested in personalized medicine projects.

Conclusions

Precision medicine projects based on targetable gene panel approximations can obtain translatable information to pediatric patients with reasonable efforts. Since the translation to the clinical practice is the main objective of these projects, limiting the number of relatively well-known biological markers will allow us to transfer similar amount of information with less economic and human effort.

The presented experience performed under limited human and financial resources, explores differences within published data. Our results support alternative ways on how to apply a pediatric precision medicine aiming the transfer of as much information as possible with the lowest possible expenses.

Additional files

Additional file 1: Table S1. Genes included in Human Comprehensive Cancer GeneRead DNAseq Targeted genes Panel (Qiagen©). (PPTX 63 kb)

Additional file 2: Table S2. Genes included in Ion Ampliseq Cancer Hot Spots v2 (Thermo Fisher Scientific©). (PPTX 46 kb)

Additional file 3: Reference database. (XLSX 21 kb)

Abbreviations

CNV: Copy number variations; MTB: Molecular Tumor Board; NGS: Next generation sequencing; PMTB: Pediatric Molecular Tumor Board; POU: Pediatric oncology units; SIOPEN: International Society of Pediatric Oncology /Europe Neuroblastoma; WES: Whole exome sequencing

Acknowledgements

Désirée Ramal has contributed in an essential way in the study development and management of the data derived from the project.
This work was supported by "Sumemos muchas manos por los niños enfermos", "Cancercare Xàvia", "Asociación Cáncer Algemesí".

Pablo Gargallo is a research Fellow of Post-MIR program IIS La Fe, Valencia Spain.
The Pediatric Hemato-Oncolgy Unit of University Hospital La Fe is a node of the European Network for Research in Pediatric Cancer (ERN-PAEDCAN).

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Authors' contributions

All authors read, corrected and approved the final manuscript. All authors are official members of "Molecular Tumor Board" in La Fe Hospital (Valencia, Spain). All the authors have contributed in an essential way obtaining data, joint analysis of the same and obtaining the conclusions in the text collected and transferred to the families (each one from the point of view of their specialty).

Funding

Funding has been obtained from private micro-donations, and these from patient associations.

Availability of data and materials

Yes. Supplementary material.

Ethics approval and consent to participate

Every patient or legal guardian, in the case, were informed adequately and signed the informed consent. The Project was approved for Ethics committee for biomedical research (La Fe Hospital, Valencia, Spain).

Consent for publication

None individual person's data is included, and therefore, not applicable.

Competing interests

The authors declare that they have no competing interests

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Received: 5 April 2019 Accepted: 21 June 2019
Published online: 02 July 2019

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