



Original Research

Systematic target actionability reviews of preclinical proof-of-concept papers to match targeted drugs to paediatric cancers



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Abstract Background: Children with cancer are in urgent need of new therapies, as approximately 25% of patients experience a relapse and 20% succumb to their disease. Moreover, the majority of survivors suffer from clinically relevant health problems. Repurposing of targeted agents developed for adult indications could provide novel therapeutic options for paediatric cancer patients. To prioritise targeted drugs for paediatric clinical development, we applied a systematic review methodology to develop a Target Actionability Review (TAR) strategy. These TARs assess the strength and completeness of published preclinical proof-of-concept (PoC) data by structured critical appraisal of and summarising the available scientific literature for a specific target (pathway) and the associated drugs in paediatric tumours.

Methods: A sensitive literature search in PubMed was performed and relevant papers were identified. For each paper, the individual experimental findings were extracted, marked for

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paediatric tumour type and categorised into nine separate PoC data modules. Each experimental finding was scored for experimental outcome and quality independently by two reviewers; discrepancies were assessed by a third reviewer and resolved by adjudication. Scores corresponding to one PoC module were merged for each tumour type and visualised in a heat map matrix in the publicly available R2 data portal [r2.amc.nl].

Results and conclusions: To test our TAR methodology, we conducted a pilot study on MDM2 and TP53. The heat map generated from analysis of 161 publications provides a rationale to support drug development in specific paediatric solid and brain tumour types. Furthermore, our review highlights tumour types where preclinical data are incomplete or lacking and for which additional preclinical testing is advisable.

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1. Introduction

Paediatric cancer remains the leading cause of disease-related death in children and adolescents [1]. However, due to its rarity (<1% of cancers [2]), the majority of cancer drug development is focused on adult malignancies [1]. In addition, a limited number of paediatric-specific clinical trials are feasible because of low patient numbers and funding at any given time. Therefore, far fewer therapies are approved for children than for adults. Paediatric oncologists may resort to off-label use of drugs approved or in clinical trials for adults, but this *ad hoc* administration outside of systematic paediatric trials precludes systematic evaluation and cannot fulfil the ethical and legal demand of safe and tested drugs for children with cancer [4]. Furthermore, paediatric tumours generally have a lower mutational burden than adult malignancies and thus fewer, yet potentially more specific, therapeutic targets [5,6]. In order to efficiently guide clinical development for a novel targeted agent, comprehensive proof-of-concept (PoC) preclinical data are essential. To this end, we have developed a systematic literature review strategy for targeted interventions in paediatric tumour types as part of the Innovative Therapies for Children with Cancer [7] Paediatric Preclinical PoC Platform (ITCC-P4), an Innovative Medicines Initiative 2 (IMI2)-funded public–private partnership between academic research institutions and pharmaceutical companies. This structured stepwise review of published literature was performed on a particular target gene or pathway and corresponding drug(s)/compound(s) across a broad panel of 16 paediatric solid and brain tumour types. Our review highlights the strength and extent of, and gaps in, the current knowledge of the drug target and associated drugs in a specific paediatric malignancy. Furthermore, it encourages additional preclinical testing in a more efficient manner. With these efforts, we provide guidance for well-informed decision-making on and prioritisation of subsequent further preclinical and clinical evaluation.

2. Methods

Two independent reviewers performed the initial stepwise systematic review process by determining whether individual published studies address one of eight preclinical and one clinical PoC modules. These modules are based on the output of the International Society of Paediatric Oncology (SIOP) Taskforce on Target Actionability and focus on aspects of tumour dependency on a specific target as well as the effects of corresponding targeted compounds on tumour growth and disease progression (see Table 1 for details on the PoC modules). Both reviewers independently extracted and appraised experimental findings from the selected papers guided by critical appraisal questions for each of the PoC modules. A third reviewer was included at Step 3 to ensure the robustness of the review and to aid in the resolution of discrepancies between the first two reviewers. The publicly available web portal R2 [r2.amc.nl] was set up for online review, appraisal adjudication and visualisation of results. The four general steps are summarised below and in Fig. 1 and an example is given in Supplementary Fig. 1.

2.1. Step 1: extensive literature search for papers on paediatric tumours of interest

PubMed is queried using specific keywords agreed upon by the initial two reviewers: [“*paediatric cancer type*” AND “*target(s)/pathway*”] and, in some cases, [“*paediatric cancer type*” AND “*drug name*”]. The scope of the TAR is limited to paediatric solid and brain tumour histologies (listed in Table 2). Based on the identified titles and abstracts, the reviewers independently decided which papers identified by the PubMed search merit inclusion in the downstream analysis. Papers addressing at least one of the corresponding critical appraisal questions for a specific PoC module (Table 1) were included; review articles were excluded. Reviewers agreed on a single list of PubMed

Table 1
Critical appraisal questions and framework for key experimental findings to summarize in TAR.

Proof-of-concept module (PoC)	Critical appraisal questions	Information to include in summaries of experimental findings
PoC 1: target/pathway activation in paediatric clinical series	<p><i>Is the target pathway active in the tumour of interest?</i></p> <p>Target/pathway evaluation in clinical series: DNA aberrations, (over)expression, methylation changes?</p> <p>Target DNA aberrations: mutation, translocation, amplification, in/del, CNV</p> <p>Percent of samples with aberrant target/pathway in clinical series</p> <p>Distribution over clinical risk groups</p> <p>Correlation to clinical outcome</p> <p>Correlation to other tumour biology</p> <p>Target expression/pathway activity compared to normal tissue, other cancers, and/or other reference tissue</p>	<p>Total size of cohort (consider only the number of patient samples, not cell lines)</p> <p>Methodology used</p> <p>Percent of samples expressing the target (and associated alterations or mutation) or with activated target pathway</p>
<p>Tumour target dependence</p> <p>PoC 2: <i>in vitro</i></p> <p>PoC 3: <i>in vivo</i></p>	<p><i>Is the tumour of interest dependent on the target or pathway for survival?</i></p> <p><i>In vitro</i></p> <p>Molecular target gene silencing in cells (RNAi, AOs, CRISPR, etc.) or ectopic expression; preferably ≥ 3 cell lines</p> <p>Phenotype analysis (apoptosis, cell viability, etc.)</p> <p>Biological effect of molecular silencing or ectopic expression of target</p> <p>Appropriate controls (use of multiple silencing tools, rescue experiments, control cell lines, etc.)</p> <p>Additional functional assays showing target or pathway dependence for mutated/translocated/amplified target genes</p> <p><i>In vivo</i></p> <p>Molecular silencing or overexpression of target gene in xenografts (inducible shRNA or expression vectors)</p> <p>Transgenic models (mice, zebrafish, etc.) for mutated/translocated/amplified target genes or for activated pathways</p>	<p><i>In vitro/in vivo</i></p> <p>Model(s)</p> <p>Methodology used</p> <p>Results of initial experiment (generally, cell viability or tumour growth)</p> <p>Rescue experiment used</p> <p>Validation (effects on apoptosis, proliferation, cell cycle, migration, gene or protein expression, etc.)</p>
<p>Sensitivity to tool compound/drug</p> <p>PoC 4: <i>in vitro</i></p> <p>PoC 5: <i>in vivo</i></p>	<p><i>Does the targeted compound reduce survival of the tumour of interest in preclinical models? ('Proof of principle': can a chemical 'tool compound' hit the target and produce the desired biological effect?) ('Proof of concept': can a drug in clinical development hit the target and produce the desired biological effect at a clinically relevant concentration(s)?)</i></p> <p><i>In vitro</i></p> <p>Preferably ≥ 4 cell lines with target dependence (preferably with ≥ 1 control cell line without target dependence)</p> <p><i>readout 1</i> Cell viability: IC₅₀, GI₅₀, LC₅₀, survival curves</p> <p><i>readout 2</i> Biological efficacy: preferably measured with pharmacodynamic (PD) assays intended for extrapolation to clinical studies</p> <p>Correlation of efficacy with tumour biology</p> <p><i>In vivo</i></p> <p>Xenografts/PDX/GEMM (both with dependency on evaluated target)</p> <p>Preferably measured with predictive biomarker to be used in clinical trial for patient selection</p> <p><i>readout 1</i> Pharmacokinetics (PK; plasma and intratumoural)</p> <p><i>readout 2</i> Pharmacodynamics in tumour: 1. target binding, 2. target inhibition, 3. pathway modulation, 4. biological effect</p> <p>PK - PD relationships: preferably use assays intended for extrapolation to clinical studies</p> <p><i>readout 3</i> Response rates and survival measures (use established, measurable tumours)</p> <p>Efficacy - PD - PK relationships</p>	<p><i>In vitro</i></p> <p>Type (established cell line or patient-derived [i.e. <i>ex vivo</i>]) and number of cell lines used [including controls]</p> <p>Drug(s) used and concentration range tested; time point(s) used to assess cell viability</p> <p>Percent of sensitive lines (IC₅₀ \leq 500 nM or clinically relevant [if known/applicable])</p> <p>Validation (effects on apoptosis, proliferation, cell cycle, migration, gene or protein expression, etc.)</p> <p><i>In vivo</i></p> <p>Model(s) (cell-line or patient-derived xenografts, transgenic mice, orthotopic v subcutaneous, etc.) and <i>n/arm</i></p> <p>Dosing schedule used</p> <p>Tumour growth inhibition and/or overall response extrapolation for each experiment</p> <p>Validation (effects on apoptosis, proliferation, cell cycle, migration, gene or protein expression, etc.)</p>
PoC 6: predictive biomarkers	<p><i>Can biological compound efficacy be determined by a specific marker in preclinical models?</i></p> <p>Evaluation of existing, validated biomarkers in PoC4 and PoC5</p> <p>Predictive biomarker (intended for extrapolation to clinical studies and patient selection)</p> <p>Efficacy biomarkers (PD markers)</p>	<p>Biomarker(s) reported</p> <p><i>In vitro/in vivo</i> correlation (include statistical values if available)</p> <p>Patient correlation (include statistical values if available)</p> <p>Patient correlation (include statistical values if available)</p>
PoC 7: resistance	<p><i>Are the mechanisms of resistance understood?</i></p> <p>(Analysed in preclinical models, use knowledge from adult studies, added observations in patient samples from trials)</p> <p>Target mutations</p> <p>Upregulation of alternative pathways</p> <p>Increased drug transporters</p> <p>Other mechanisms</p>	<p>Model(s) (<i>in vitro/in vivo</i>)</p> <p>Methodology</p> <p>Resistance reported and drug concentration / validation (if applicable)</p>

PoC 8: combinations	<p><i>Are synergistic combinations with other drugs/compounds established?</i></p> <p>Rational combinations: based on pathway knowledge and/or resistance observations from PoC7</p> <p>Compound/drug + cytotoxics</p> <p>Compound/drug + targeted compound</p>	<p>Model(s) (<i>in vitro/in vivo</i>)</p> <p>Methodology for combination (combination of multiple drugs, combination of drug plus knockdown, etc.)</p> <p>Drug(s) used and concentration range tested; time point(s)</p> <p>Drug(s) used and concentration range tested; time point(s)</p> <p>Results (Combination index [CI]/method of determining combination effect, percent of models showing synergism)</p> <p>Validation (effects on apoptosis, proliferation, cell cycle, migration, gene or protein expression, etc.)</p>
PoC 9: clinical evaluation	<p><i>Can the targeted compound safely be administered to children with cancer? ('phase I')</i></p> <p>Has a formal phase I trial been conducted with a targeted compound in children with cancer?</p> <p>Has a recommended dose been established for single drug use?</p> <p>Has a recommended dose been established for use in combinations in standard of care (SOC)??</p> <p><i>Does the targeted compound show efficacy (clinical or biological) in relapsed/refractory disease? ('phase II')</i></p> <p>Has a formal phase II trial been performed with a targeted compound in children with cancer?</p> <p>In which diseases has efficacy been investigated?</p> <p>In which stage of disease (Relapsed/refractory? Treatment-naïve?)</p> <p>Were trials done with single drug or in combinations?</p> <p>Has 'biological efficacy' (PD biomarkers) been shown?</p> <p><i>Does the targeted compound add benefit to the standard-of-care treatment? ('phase III')</i></p> <p>See EBM critical appraisal checklists for 'therapeutic interventions'*</p>	<p>Number of patients included in the trial and tumour types considered</p> <p>Study design (phase, type of design [open-label, randomized, controlled, other])</p> <p>Toxicity profile</p> <p>Recommended phase II dose, if applicable</p> <p>Efficacy signal observed (ORR, CR, PR, SD or PD), if applicable</p>

*<http://www.cebm.net/critical-appraisal/>

IDs (PMIDs) to upload to the R2 data application, which automatically links the PMIDs to the full texts of these relevant papers.

2.2. Step 2: critical evaluation and scoring of papers

Papers were critically read and appraised by the two reviewers independently, guided by the critical appraisal questions for each PoC module (Tables 1, 3 and 4). Both reviewers identified the tumour type(s) and PoC data modules addressed in each study, then summarised the key experimental findings for each PoC and entered these in the R2 TAR platform. To ensure a standardised literature review, a scoring system was defined based on two parameters: 'experimental quality' and 'experimental outcome'. 'Experimental quality' is a measure of the quality of reported findings, with scores ranging from 1 to 3, which are determined by experimental methods, number of samples and controls and additional variables (Table 3); 'experimental outcome' (ranging from -3 to +3) scores the extent of the reported results in pre-defined quantitative categories based on whether the study results support targeting a particular pathway in a specific tumour type (Table 4). Both reviewers scored each key experimental finding for the two parameters independently from each other (Fig. 1). The resultant data summary and accompanying scores for both reviewers constitute one 'data entry' and are stored in the R2 TAR platform (Fig. 3D).

2.3. Step 3: reviewer adjudication

The R2 TAR platform detected and highlighted scoring discrepancies between the two reviewers who then discussed the discordant PoC modules to reach a consensus in their scores. Subsequently, a third reviewer,

blinded to the previous scores in R2, independently assessed the highlighted papers with scoring conflicts to add another layer of unbiased review. In the event that the third reviewer disagreed with the adjudicated scores of the first two reviewers, the three reviewers discussed and came to a consensus, resulting in one set of finalised scores and experimental findings, which were updated in R2.

2.4. Step 4: generation of finalised heat map

The adjudicated experimental outcome and quality scores for each data entry are multiplied by R2 in order to better separate higher quality data from lower quality. Multiplication of both scores results in scores ranging from -9 to +9. The application subsequently averages all available multiplied scores into one 'appraisal score' for each PoC module within a specific tumour type, with the direction and magnitude indicating the strength of a positive or negative result. Papers with high-quality methodology are thus weighted in this appraisal score.

A heat map is generated in R2 (Fig. 3C) from the appraisal scores in a gradient colour code, with yellow indicating negative results and blue signifying positive results. Hovering over a square representing the average appraisal score for a POC module within a specific tumour type causes a box to pop up and display the average and median scores along with the number of papers analysed for that particular module. The number of papers can also be displayed within each box of the heat map. Clicking on a square in the heat map will display the list of papers included in that specific PoC module along with the accompanying summary of the experimental findings, the scores for experimental quality and outcome and direct links to PubMed (Fig. 3D).

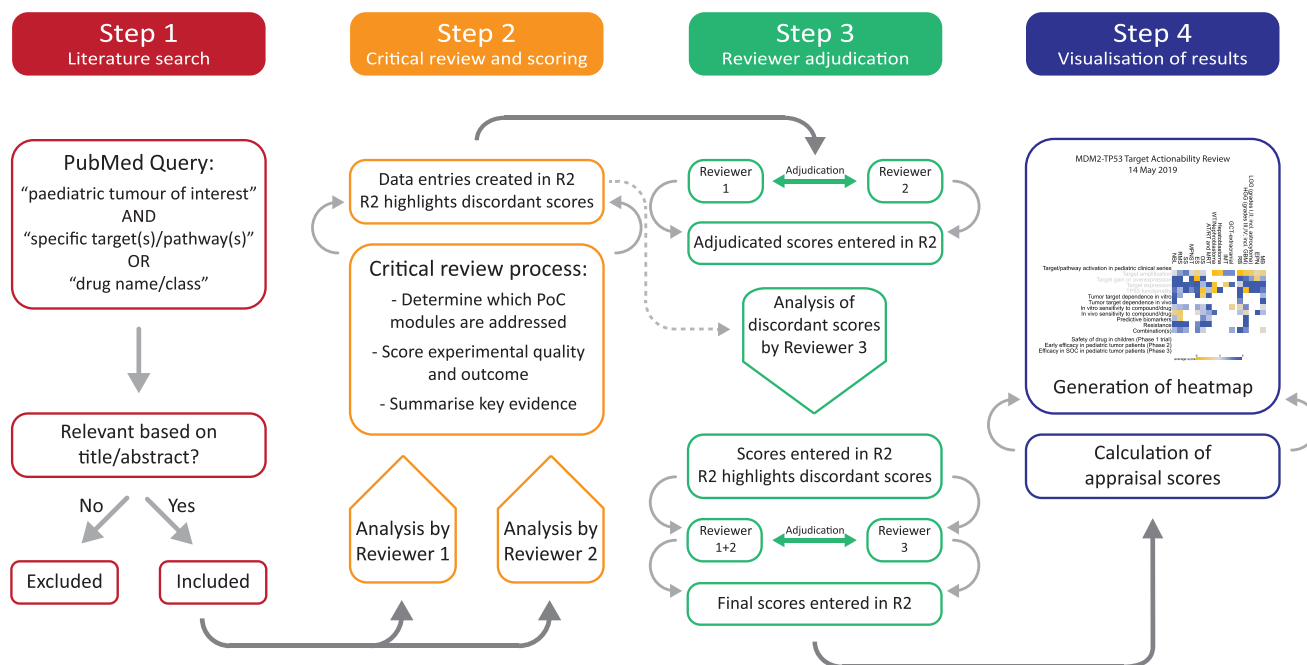


Fig. 1. Overview of Target Actionability Review (TAR) methodology.

3. Results

In order to test our methodology and to identify and implement any modifications deemed necessary through an iterative approach, we conducted a pilot review on MDM2 and the *TP53* gene. The p53 pathway is well characterised for its role in tumorigenesis and disease progression across different types of cancer. MDM2 (also called HDM2), a transcriptional target of the tumour suppressor p53, is an E3 ubiquitin ligase, which negatively regulates p53 through blocking its transcriptional activity, targeting it for proteasomal degradation and by inhibiting *TP53* mRNA translation [8]. *MDM2* amplification, copy number variations (CNVs) and overexpression promote tumour growth by disrupting the balance between MDM2 and p53 function [9]. Therefore, the MDM2–p53 interaction is an attractive therapeutic target and MDM2 inhibition in particular has been the focus of several drug development efforts. As mutations in p53, which are less frequent in childhood tumours, impair the transcription of its target genes (including *MDM2*), the activity of most MDM2 inhibitors is dependent on wild type *TP53* [10,11]. Evaluation of first-generation MDM2 inhibitors nutlin-3 and RG7112 demonstrated poor or highly variable bioavailability and high toxicity in adult patients. Consequently, the clinical focus has shifted to next-generation inhibitors, including idasanutlin (RG7388) and DS-3032b (Supplementary Table S1) [9,12,13].

The search terms ["paediatric cancer type" AND (*MDM2* OR *HDM2*)] and ["paediatric cancer type" AND *nutlin*] identified 726 unique papers (search date:

11 March 2019), 161 (22%) of which met our inclusion criteria (Fig. 2). After independent evaluation of these papers by the first two reviewers, 343 data entries were created in R2; of these, 106 (31%) were scored differently by the two reviewers. Discrepancies in 37 of the 106 data entries that were initially discordant between the first two reviewers (35%) were noted after the third review; all discrepancies were resolved after the final adjudication. No papers or entries were excluded during reviewer discussions and the final number of adjudicated data entries remained 338.

Due to the multiple possible mechanisms of MDM2–p53 pathway dysregulation, the 'target/pathway pattern module' (PoC 1; Tables 1 and 5) was further divided into subcategories to differentiate among (a) *MDM2* amplification, (b) gain (either whole chromosome or focal) or overexpression and (c) expression (generally as determined by immunohistochemistry). In addition, the mutational status of *TP53* reported in 12 recent studies subjecting paediatric tumour samples to next-generation sequencing was recorded as part of PoC module 1 (d). As *TP53* mutations are generally thought to be inactivating and thereby abrogating the need for MDM2-mediated p53 inactivation, 'experimental outcome' scores were inverted for PoC 1d (Table 5).

Neuroblastoma (NBL) was the most represented childhood cancer in the TAR with 45 selected papers generating 88 individual data entries, followed by osteosarcoma (OS; 28 papers/38 data entries), rhabdomyosarcoma (RMS; 25/40) and Ewing's sarcoma (ES; 16/26) (Fig. 3A and B). In the brain tumour space, high-grade glioma (HGG, WHO grades III and IV) was the focus of 15 papers (25 data entries) whereas 31 data entries were

Table 2

Tumour types included in TAR search criteria.

Tumour histology	Subtypes	Reference
Neuroblastoma (NBL)	<i>MYCN</i> -amplified Non- <i>MYCN</i> -amplified	
Rhabdomyosarcoma (RMS)	Alveolar (aRMS) <i>PAX3</i> -fusion positive <i>PAX7</i> -fusion positive Embryonal <i>RAS</i> mutant <i>RAS</i> wild-type	[53,54]
Synovial sarcoma (SS) Malignant peripheral nerve sheath tumour (MPNST) Ewing's sarcoma (ES)	FET-ETS FET-ETS-plus Non-FET-ETS	[55]
Osteosarcoma (OS) Atypical teratoid/rhabdoid tumour + malignant rhabdoid tumour (AT/RT + MRT)	TYR SHH MYC Extracranial rhabdoid	
Wilms tumours/nephroblastoma (WT) Hepatoblastoma (HB) Inflammatory myofibroblastic tumour (IMT) Extracranial germ cell tumour (GCT) Retinoblastoma (RB)		
Low-grade glioma (WHO grades I and II) (LGG) High-grade glioma (WHO grades III and IV) (HGG)	K27M mutant G34 mutant MYCN RTK NOS	[56]
Diffuse intrinsic pontine glioma (DIPG) Ependymoma (EPN)	ST-EPN-RELA ST-EPN-YAP1 PF-EPN-A PF-EPN-B	[57,58]
Medulloblastoma (MB)	WNT SHH - p53 wild-type SHH - p53 mutant Group 3 Group 4	[59,60]

These subtypes were defined during expert panels of the ITCC-P4 consortium. Brain tumour histologies are shaded in grey.

MYCN-amplified: NBL with ≥ 8 copies of *MYCN*; *PAX3*-fusion positive: RMS with *PAX3* fusions, most common *PAX3-FOXO1*; *PAX7*-fusion positive: RMS with *PAX7* fusions, most common *PAX7-FOXO1*; *RAS* mutant: RMS with mutations in *NRAS*, *HRAS* or *KRAS*; FET-ETS: fusion between FET family member and ETS family member proteins and no other alterations; FET-ETS plus: FET-ETS fusion ES with *STAG2* and/or *TP53* and/or *CDKN2A* alterations; non-FET-ETS: Ewing-like sarcoma (typically with *BCOR*, *CIC* or *NFACT2* fusions); TYR subgroup: high expression of the TYR gene; SHH subgroup: extensive sonic hedgehog (SHH) signalling; MYC subgroup: characterised by overexpression of the MYC gene; K27M mutant: gliomas with a somatic K27M histone (H3) mutation; G34 mutant: gliomas with a somatic G34 R/V histone (H3.3) mutation; MYCN: gliomas enriched for MYCN/MYC amplifications; RTK: gliomas with *PDGFRA* amplifications; NOS: not otherwise specified; ST-EPN-RELA: supratentorial EPN with *RELA* fusions; ST-EPN-YAP1: supratentorial EPNs with *YAP1* fusions; PF-EPN-A: posterior fossa EPN with a largely balanced chromosomal profile; PF-EPN-B: posterior fossa EPNs with a high degree of genomic instability; WNT: MB primarily driven by Wingless signalling pathways; SHH: MB primarily driven by sonic hedgehog signalling pathways, with or without *TP53* mutation; Group 3: no unifying underlying pathway known, worst prognosis; Group 4: no unifying underlying mechanism known.

Table 3
Rubric for scoring experimental quality.

Proof-of-concept module (PoC)	Description	Scoring and criteria
PoC 1: target/pathway activation in paediatric clinical series	Number of paediatric samples Type of analysis	3 $n \geq 20$ paediatric patient samples ≥ 2 different methods OR next-generation sequencing
		2 $20 > n > 10$ paediatric patient samples ≥ 1 reliable method
		1 $n \leq 10$ paediatric patient samples 1 method
PoC 2: tumour target dependence <i>in vitro</i>	Methodology Tumour cell viability Biological pathway readout	3 Different methods to alter target expression in ≥ 3 cell lines Phenotypic analysis of knockdown
		2 Single method to alter target expression in < 3 cell lines Questionable alteration of gene expression
		1 Questionable alteration of gene expression
PoC 3: tumour target dependence <i>in vivo</i>	Model used Tumour formation/growth Biological pathway readout	3 Transgenic mouse model or ≥ 2 different xenografts with appropriate controls and/or different methods of genetic modification <i>in vivo</i> (shRNA/CRISPR)
		2 ≥ 2 different xenografts without appropriate control 1 xenograft model without appropriate control
		1 1 xenograft model without appropriate control
PoC 4: <i>in vitro</i> sensitivity to compound/drug	Number of cell lines Measurement of PD markers and/or phenotypic response	3 5+ cell lines + ≥ 2 appropriate controls; validation
		2 2–5 cell lines + ≥ 1 appropriate controls; validation 1 1 cell line and/or lack of control and/or validation
PoC 5: <i>in vivo</i> activity of compound/drug	Number and type of models used Measurement of PD markers and/or phenotypic response	3 ≥ 2 xenograft models or 1 transgenic mouse model with appropriate control; treatment with clinically relevant dose; validation
		2 1 xenograft model with appropriate control; treatment with clinically relevant dose; validation
		1 1 xenograft model OR use of supra-clinical dose levels; no appropriate control or validation
PoC 6: predictive biomarkers	Confirmation of correlation Patient selection	3 Correlation molecularly confirmed in ≥ 2 models (e.g. silencing, overexpression, etc.); patient selection
		2 Correlation confirmed in one model
		1 Correlation not confirmed
PoC 7: resistance	Mechanism of resistance Molecular analysis Method to overcome resistance	3 Reported resistance and comprehensive analysis and reversing/overcoming resistance
		2 Reported resistance and analysis of molecular changes underlying/due to resistance
		1 Only reporting resistance
PoC 8: combinations	Concentrations tested <i>In vitro</i> combination index values <i>In vivo</i> combination	3 > 4 concentrations of each compound are tested and combination index values calculated; combination evaluated <i>in vivo</i>
		2 1–4 concentrations of each compound are tested and combination index values calculated; with or without evaluation of combination <i>in vivo</i>
		1 Only one concentration of each compound is tested; no evaluation of combination <i>in vivo</i>

generated from 13 medulloblastoma (MB) papers. Ependymoma (EPN) and low-grade glioma (LGG, WHO grades I and II) were studied in 10 and 8 papers (11 and 10 data entries), respectively. The majority of papers focused on one tumour type; however, 13 of the 162 included papers (8%) investigated multiple histologies.

Overall, PoC 1 was the module most frequently addressed by the included studies (Fig. 3A and B). *MDM2* amplification and chromosomal gains were relatively frequent in RMS, with up to 32% of cases in one study [14]. While the incidence of *MDM2* alterations were similar between the alveolar and embryonal RMS subtypes [15,16], *TP53* mutations usually occurred in PAX fusion negative RMS but in generally less than 15% of those cohorts [17,18]. In OS, *MDM2* amplifications could be detected in up to 83% of patient samples

in individual reports and were more frequent in the parosteal subtype [19,20]. However, *TP53* mutations were also prevalent in this histology, with 50–75% of patient tumour samples having at least one inactivating aberration [6,21]. The prevalence of *MDM2* alterations in synovial sarcoma (SS), malignant peripheral nerve sheath tumour (MPNST) and ES is ambiguous due to the limited number of included studies that often contradicted each other. Most of the studies on cohorts of paediatric HGG, LGG and MB did not report *MDM2* amplification, but rather a focal or chromosomal gain or overexpression of *MDM2* (Fig. 3). Interestingly, while *MDM2* structural abnormalities were absent in EPN patient samples, *MDM2* and p53 were routinely detected by immunohistochemistry.

The finalised heat map (Fig. 3C) visualisation in R2 of the merged appraisal scores in POC modules per

Table 4
Rubric for scoring experimental outcomes.

Proof-of-concept module (PoC)	Description	Scoring and criteria
PoC 1: target/pathway activation in paediatric clinical series	Prevalence of target/pathway in cohort	3 More than 10% of cohort 1 Between 2 and 10% -3 ≤2% of cohort
PoC 2: tumour target dependence <i>in vitro</i>	Level of dependency and phenotypic recapitulation	3 Full dependency (>75% cell death OR transformation) 1 Partial dependency (<75% cell death OR altered growth) -3 No dependency
PoC 3: tumour target dependence <i>in vivo</i>	Level of dependency and phenotypic recapitulation	3 Full dependency (CR) after knockdown/knockout or transformation in GEMM 1 Partial dependency (<75% response) -3 No dependency
PoC 4: <i>in vitro</i> sensitivity to compound/drug	IC ₅₀ observed after 72 h exposure	3 IC ₅₀ < 500 nM or ≤ clinically relevant concentration ^a 1 IC ₅₀ = 500–1500 nM -1 IC ₅₀ > 1500 nM -3 No activity (IC ₅₀ > 10 μM)
PoC 5: <i>in vivo</i> activity of compound/drug	<i>In vivo</i> tumour response	3 Response comparable to PR/CR 1 Response comparable to SD -1 Very minor response (between SD and PD, slight TGI) -3 No activity or clear PD, growth comparable to control
PoC 6: predictive biomarkers	Correlation of biomarker status with anti-cancer activity of a targeted drug <i>in vitro/in vivo</i>	3 Strong correlation (presence of biomarker results in significantly different drug response) 1 Moderate correlation (presence of biomarker results in different drug response, not significant) -3 No correlation (presence of biomarker does not correlate with drug response)
PoC 7: resistance	Reported resistance with drug exposure	3 Resistance reported at clinically relevant concentration/dose and identification/description of mechanism 1 Resistance reported with no mechanism
PoC 8: combinations	Synergy in combination testing at clinically relevant dosages in relevant <i>in vitro</i> and/or <i>in vivo</i> models	3 Strong synergy reported – combination index (CI) < 0.5 1 Moderate synergy/additive effect - CI 0.5–0.9 -1 Very minor synergy/additive effect observed - CI 0.9–1.1 -3 No combination benefit
PoC 9: clinical trials	Phase I	3 Toxicity profile acceptable ^b , RP2D identified and early efficacy observed 1 DLT observed with still acceptable safety and no efficacy observed -3 Toxicity profile not acceptable
	Phase II	3 Efficacy observed greater than historical ORR, DoR and/or PFS and acceptable toxicity 1 Limited efficacy observed above the historical ORR, DoR and/or PFS and acceptable toxicity -3 No efficacy observed and/or unacceptable toxicity
	Phase III	3 Added efficacy over SOC in appropriate pivotal trial with acceptable benefit/risk profile New drug now part of SOC 1 Added efficacy over SOC but new agent not part of SOC, due to trial design issues and/or benefit/risk assessment -3 Insufficient efficacy in pivotal trial

CR: complete regression, disappearance of tumour; PR: partial regression, ≥30% decrease of tumour volume; SD: stable disease, neither PR nor PD criteria met; PD: progressive disease, ≥20% increase of tumour volume; TGI: tumour growth inhibition; criteria based on RECIST criteria [62]. RP2D: recommended phase 2 dose; DLT: dose-limiting toxicity; ORR: overall response rate; DoR: duration of response; PFS: progression-free survival; SOC: standard-of-care.

NB: if publications did not address the experimental outcomes according to these criteria, the outcomes were estimated and scored based on this table.

^a Clinically relevant concentration: the dose that corresponds to the maximum plasma concentrations reached in patients without signs of toxicity.

^b Toxicity profile is acceptable if adverse events are not life-threatening (no higher than Grade 3 based on the Common Terminology Criteria for Adverse Events) [61].

tumour type revealed a general lack of evidence for molecular target validation in modules PoC 2 and PoC 3 with three papers for each retinoblastoma (RB) and MB and one for each RMS, ES, OS and Wilms tumour (WT) (Fig. 3B and C). The only tumour type with five papers was NBL, but only one performed target

validation in an *in vivo* setting. Conversely, the TAR identified 55 publications focused on *in vitro* and *in vivo* sensitivity testing of MDM2 inhibitors (PoC 4 and 5); these studies, combined with the extensive characterisation of the MDM2-TP53 pathway in adult malignancies, may reduce the need to further validate MDM2

as a driver of tumourigenesis in paediatric cancers (PoC 2 and 3). The majority of studies (80%) focused on the nutlin class of drugs. For NBL, 19 studies examined MDM2 inhibition exclusively *in vitro*, while nine studies were conducted both *in vitro* and *in vivo*. Idasanutlin (RG7388) was determined to be the most potent MDM2 inhibitor in this indication and induced p53 pathway activation, cell cycle arrest and apoptosis [22]. Similarly, both RMS and OS cell lines were reported to be sensitive to nutlins, MK-8242 and/or MI-63; however, these particular inhibitors are not clinically viable options (Supplementary Table 1). Although OS may also be a promising indication, the high prevalence of *TP53* mutations [23] may preclude use of MDM2 inhibitors in patients (Fig. 3C).

Only three of nine papers evaluating MDM2 inhibitors in NBL cell line-derived xenograft (CDX) models reported anti-tumour activity; the endpoints used in these studies ranged from a reduction in tumour burden and weight with idasanutlin treatment or increased animal survival following treatment with either DS-3032b or RO6839921 [24–26]. Tumour growth, albeit delayed when compared to control animals, was still evident in the various treatment groups. RMS xenograft models were tested for sensitivity to MDM2 inhibitors in three publications and two reported that one or two xenograft models responded to

either RG7112 (1/7 models with complete response) [27] or MK-8242 (1/5 models with maintained complete response; 1/5 with stable disease) [28]. Studies using OS mouse models were contradictory, with reported responses to MDM2 inhibition ranging from progressive disease [27–29] to a dose-dependent decrease in tumour volume leading to tumour regression [30,31]. Importantly, NBL, RMS and OS cell lines acquired mutations in *TP53* following long-term nutlin treatment, resulting in drug resistance (PoC 7) [32,33]. Combination of an MDM2 inhibitor with chemotherapy or other drugs was studied extensively in NBL (with 23 papers included in PoC 8), frequently resulting in enhanced tumour reductions both *in vitro* and *in vivo*.

4. Discussion

The ITCC-P4, a partnership between academia and the pharmaceutical industry, aims to accelerate science-driven paediatric drug development with the goal of introducing or repurposing (from adult indications) effective novel treatments and prioritising their clinical development for children dying of rare cancers [34]. To support such prioritisation, we developed a systematic literature review methodology of preclinical PoC studies to assess the potential actionability of a target gene/pathway in paediatric solid and brain tumours in a structured and reproducible manner. Our unique TAR strategy can be used to identify mechanism-of-action-based matches between targeted anti-cancer drugs and specific cancer subtypes. Matches with strong and complete preclinical PoC may have a higher likelihood of response to treatment and could support clinical trial design. In addition, the TAR reveals gaps in the current preclinical PoC knowledge, allowing for more efficient and focused planning of additional preclinical evaluation. This type of methodology has not been applied to the paediatric cancer population; moreover, there are limited examples of systematically evaluating potential targets through review of existing literature for adult indications outside of the Cochrane Reviews [35]. Our review supports clinical evaluation of MDM2-targeting compounds in NBL, RMS and HGG; however, combination of an MDM2 inhibitor plus chemotherapy would likely be most beneficial, in order to circumvent acquired resistance to MDM2 inhibition [32,36]. Furthermore, our TAR demonstrates that while *MDM2* is amplified and/or expressed in OS patient samples, the prevalence of *TP53* mutations cautions against moving forward with MDM2 inhibitors in this indication.

In this pilot TAR, we focussed primarily on the currently available (pre)clinical knowledge on MDM2 and its inhibitors. *TP53* was, in a more limited way, added to this TAR, because of its well-known status as a biomarker for most MDM2 inhibitors. Other members of this pathway, such as p14ARF (encoded by *CDKN2A*) and MDM4, were not included. MDM4

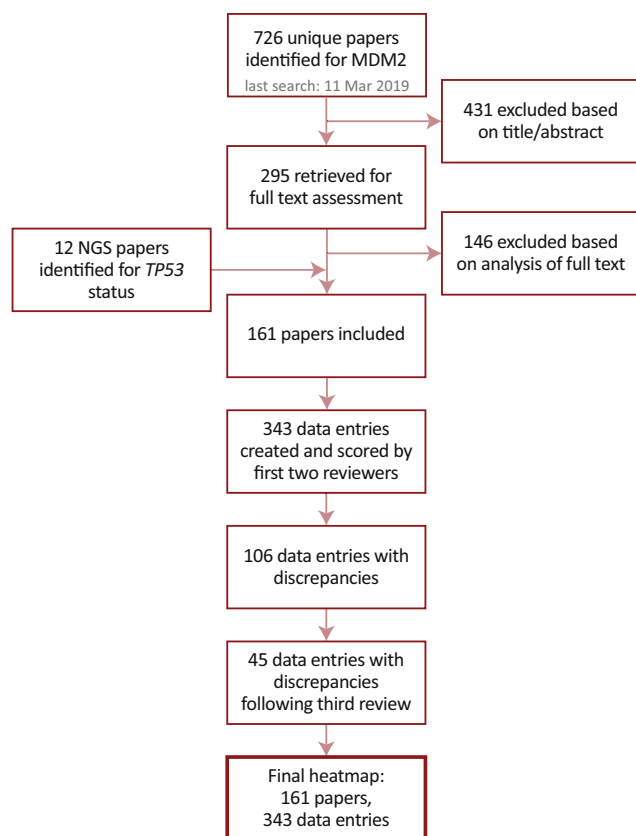


Fig. 2. Study selection process for the pilot MDM2-*TP53* TAR.

Table 5
Scoring addendum for PoC1 for the MDM2-TP53 TAR.

Proof-of-concept module (PoC)	Description	Scoring and criteria	
PoC 1: target/pathway activation in paediatric clinical series (a)	<i>MDM2/HDM2</i> amplification	3	More than 10% of cohort with amplification
		1	Between 2% and 10% of cohort with amplification
		-3	≤2% of cohort with amplification
PoC 1: target/pathway activation in paediatric clinical series (b)	(Chromosomal) gain or overexpression (OE) of <i>MDM2/HDM2</i>	3	More than 10% of cohort with gain/OE
		1	Between 2% and 10% of cohort with gain/OE
		-3	≤2% of cohort with gain/OE
PoC 1: target/pathway activation in paediatric clinical series (c)	Expression of <i>MDM2/HDM2</i> (generally, as determined by immunohistochemistry)	3	More than 10% of cohort positive for <i>MDM2</i>
		1	Between 2% and 10% of cohort positive for <i>MDM2</i>
		-3	≤2% of cohort positive for <i>MDM2</i>
PoC 1: target/pathway activation in paediatric clinical series (d)	<i>TP53</i> mutation status	3	≤2% of cohort with mutant <i>TP53</i> ^a
		1	Between 2% and 10% of cohort with mutant <i>TP53</i> ^a
		-3	More than 10% of cohort with mutant <i>TP53</i> ^a

Amplification: >8 copies, based on next-generation sequencing (NGS) techniques, array CGH, FISH or Southern blotting; *gain*: 2,5–8 copies, based on NGS techniques, array CGH, FISH or Southern blotting; *overexpression*: z-score >2 in the related cohort. If definitions are not clearly mentioned in papers, it is assumed that the authors used similar definitions.

^a *TP53* structural variations were also considered as mutant *TP53*.

overexpression was previously reported as a potential resistance mechanism after MDM2 inhibition [37–39] and combined inhibition might be beneficial [37,40]. However, neither this resistance mechanism nor the combined targeting was found within the papers included in this TAR, indicating that this interaction is not (sufficiently) studied in paediatric cancer and that further research is necessary.

Studies in which patient cohorts comprised both children (≤18 years) and adults as a group rather than two separate entities were included in PoC 1 of this TAR. Data from adult patients may inflate the actual occurrence of an aberration in paediatric tumours, as the overall mutational burden is generally much lower in children [5,6]. Conversely, several papers were excluded from our analysis as patient age was not reported [41–44] and thus our summary may not fully capture the genetic landscape in these indications. Many publications (primarily in the brain tumour space) identified during the literature search used cells derived from adult patient samples [45,46] and were subsequently removed from the TAR unless paediatric models were also included. Furthermore, while many paediatric tumour histologies are restricted to children, some cancers affect young adults well into their 20s and 30s [47,48]; moreover, some “paediatric” histologies (mainly types of sarcoma) also occur in adults over 40 years of age [49–51]. Therefore, it is pertinent to determine inclusion/exclusion status for reports where patient age is unclear or adult data are included on a case-by-case basis while maintaining the same degree of rigor.

Despite these limitations, this pilot TAR provides the most comprehensive overview to date of available pre-clinical data concerning targeting MDM2 in paediatric cancer. This TAR also highlighted a lack of studies investigating the role of the MDM2-*TP53* pathway in

paediatric tumorigenesis in preclinical models, identifying predictive biomarkers for tumour response and describing resistance mechanisms in most indications. Moreover, 45% of the studies were published before 2010; only three papers describe novel, robust methods (e.g. CRISPR) and none described newer model systems like organoids. The MDM2-*TP53* TAR demonstrated that NBL, RMS and OS were more frequently studied, accounting for 54% of the included papers. It is important to note that for some very rare tumour types, such as desmoplastic small round cell tumours, preclinical models and results from associated testing are lacking.

Furthermore, advances in diagnostic testing and molecular characterisation are helping to define new subclassifications of paediatric tumour types, especially in the brain tumour space [52]. In the R2 platform, we included some of these newly defined subclassifications of paediatric tumour types (R2 setting ‘Diseases: extensive’). Only 5% of the papers on MDM2 could clearly be assigned to a subtype, as opposed to 27% of the papers on *TP53* mutation status, likely due to the date of publication. These low numbers of subtype-specific data and the differences across subclassifications (e.g. *TP53* mutation status across all MB subtypes) demonstrate that tumour subtypes should be clearly indicated in future publications to better inform preclinical testing and/or clinical decisions.

Overall, the heat map generated from the MDM2-*TP53* TAR revealed a striking absence of published clinical trial results investigating drugs against MDM2 in a paediatric setting (Fig. 3C). This is most likely a reflection of the past clinical development landscape, where adult indications are the focus; in addition, the limited number of paediatric patient controls how many clinical trials can be performed. Moreover, this TAR clearly outlines the lack of preclinical data for specific indications (such as MPNST rhabdoid tumours,

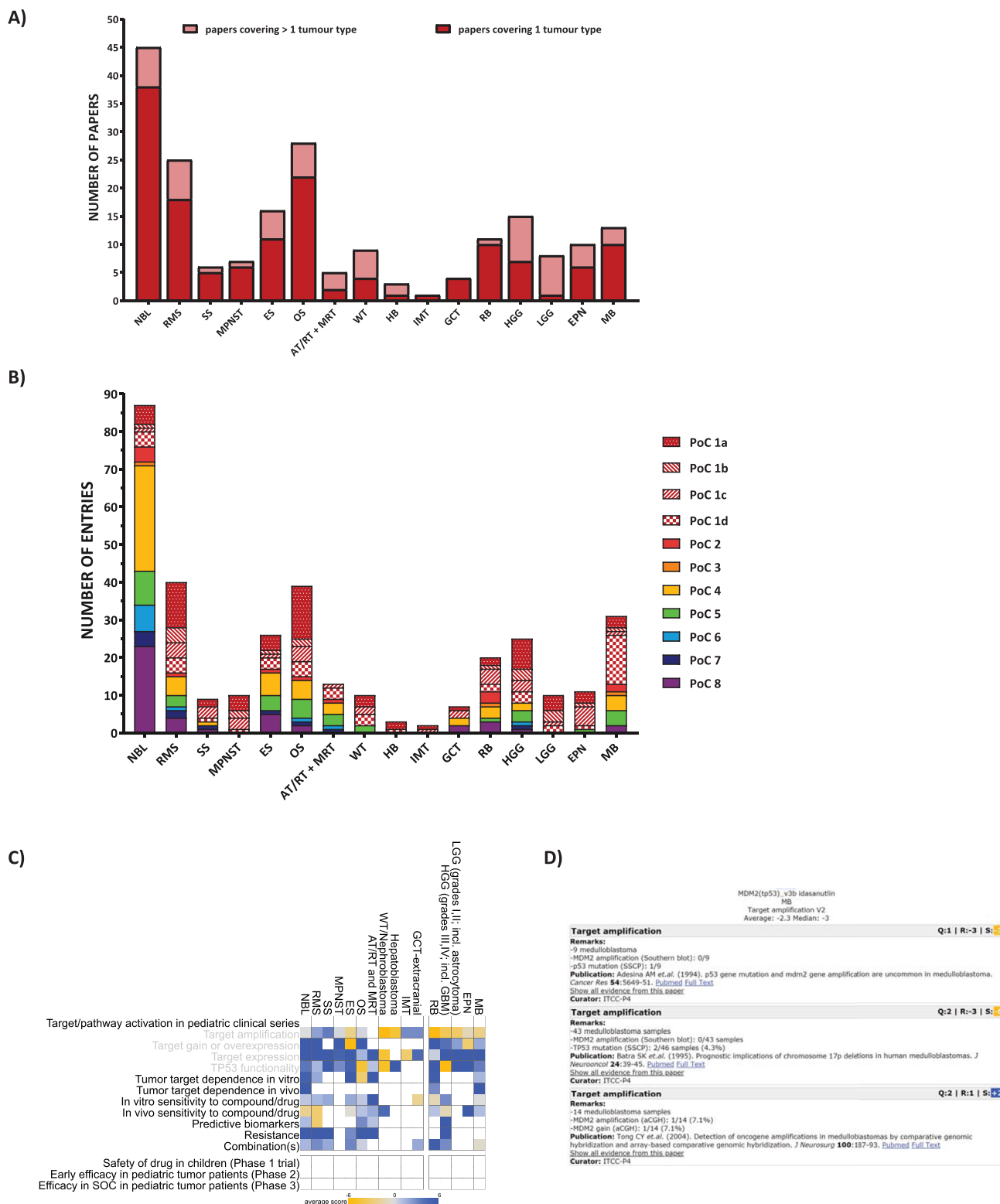


Fig. 3. Results of the MDM2-TP53 pilot TAR. The 161 papers included in the TAR visualised as a function of (A) the tumour types and (B) the PoC modules addressed. Data entries created from these studies were used to generate a heat map summary, with tumour types along the top of the grid and PoC modules along the side. (D) An example of the data entry display from the R2 platform. Here, data entries pertaining to PoC 1a (*MDM2* amplification) in medulloblastoma patient samples are shown. PoC 1a: *MDM2/HDM2* amplification; PoC 1b: (chromosomal) gain or overexpression of *MDM2/HDM2*; PoC 1c: *MDM2/HDM2* expression; PoC 1d: *TP53* mutational status.

hepatoblastoma and IMT, among others) and thus illustrates the need for additional preclinical evaluations in specific paediatric tumour types prior to moving forward with clinical development plans. Furthermore, this TAR may indirectly encourage development of more paediatric-specific preclinical models for under-represented histologies.

The TAR, including study scores, key data summaries and source information, is publicly available through R2 [r2.amc.nl]. Independent investigators will therefore be able access the data in its entirety in order to use it to support additional preclinical or clinical evaluation. The TAR points to specific indications (namely, NBL, RMS and HGG) that may benefit from MDM2-targeted therapy as it delivers a comprehensive, structured and critically appraised overview of the available preclinical evidence of actionability of a drug target in paediatric cancers. The TAR methodology and public access to existing TARs may also prove useful in the era of personalised medicine, especially in the context of molecular tumour boards, to aid in the development of patient-specific clinical strategies. However, the decision to use a targeted drug in paediatric cancer patients also depends on factors not included in the TAR, such as tumour aggressiveness, patient prognosis, other available therapies and setting (e.g. individual patient decision or drug development program planning).

It is important to continuously update the TARs to maintain the most current information within the R2 platform. This will require the near constant availability of at least three reviewers familiar with the methodology, a challenge common to most systematic literature reviews of this nature. We will conduct five additional TARs during the initial ITCC-P4 project term and aim to establish a plan for long-term sustainability of this novel tool. Finally, it will be interesting to prospectively assess the predictive value for clinical success of our preclinical PoC-based assessment of ‘target actionability’ as we see increasing numbers of clinical trials designed for various targeted anti-cancer drugs.

Contributors

HNC and JJM initiated the concept of systematic reviews of target actionability. NAS, GB, CDL, AR, TFE, JJM and HNC designed the methodology. GV, SMP and LFS provided critical input on the methodology. NAS and GB were the primary reviewers. CDL was the third reviewer. AR, JJM and HNC guided the reviewing process. JK implemented the TAR methodology and heat map visualisation in the R2 platform. NAS and CDL drafted the manuscript. AR, TFE, JJM, JK and HNC provided additional input to the manuscript. DTWJ, GV, LFS and SMP critically reviewed the manuscript.

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Conflict of interest statement

CDL and LFS are full-time employees of Eli Lilly and Company. GB, AR and HNC are full-time employees of Hoffmann-La Roche.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejca.2020.01.027>.

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