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Review

Target Actionability Review: a systematic evaluation of replication stress as a therapeutic target for paediatric solid malignancies



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Abstract Background: Owing to the high numbers of paediatric cancer-related deaths, advances in therapeutic options for childhood cancer is a heavily studied field, especially over the past decade. Classical chemotherapy offers some therapeutic benefit but has proven long-term complications in survivors, and there is an urgent need to identify novel target-

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driven therapies. Replication stress is a major cause of genomic instability in cancer, triggering the stalling of the replication fork. Failure of molecular response by DNA damage checkpoints, DNA repair mechanisms and restarting the replication forks can exacerbate replication stress and initiate cell death pathways, thus presenting as a novel therapeutic target. To bridge the gap between preclinical evidence and clinical utility thereof, we apply the literature-driven systematic target actionability review methodology to published proof-of-concept (PoC) data related to the process of replication stress.

Methods: A meticulous PubMed literature search was performed to gather replication stress-related articles (published between 2014 and 2021) across 16 different paediatric solid tumour types. Articles that fulfilled inclusion criteria were uploaded into the R2 informatics platform [r2.amc.nl] and assessed by critical appraisal. Key evidence based on nine pre-established PoC modules was summarised, and scores based on the quality and outcome of each study were assigned by two separate reviewers. Articles with discordant modules/scores were re-scored by a third independent reviewer, and a final consensus score was agreed upon by adjudication between all three reviewers. To visualise the final scores, an interactive heatmap summarising the evidence and scores associated with each PoC module across all, including paediatric tumour types, were generated.

Results and conclusions: : 145 publications related to targeting replication stress in paediatric tumours were systematically reviewed with an emphasis on DNA repair pathways and cell cycle checkpoint control. Although various targets in these pathways have been studied in these diseases to different extents, the results of this extensive literature search show that ATR, CHK1, PARP or WEE1 are the most promising targets using either single agents or in combination with chemotherapy or radiotherapy in neuroblastoma, osteosarcoma, high-grade glioma or medulloblastoma. Targeting these pathways in other paediatric malignancies may work as well, but here, the evidence was more limited. The evidence for other targets (such as ATM and DNA-PK) was also limited but showed promising results in some malignancies and requires more studies in other tumour types. Overall, we have created an extensive overview of targeting replication stress across 16 paediatric tumour types, which can be explored using the interactive heatmap on the R2 target actionability review platform [https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?option=imi2_targetmap_v1].

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1. Introduction

Despite advances in cancer therapies over the last decades, paediatric cancer remains the leading cause of disease-related death in children and adolescents, and treating these malignancies remains a major challenge [1]. Overall, less than 1% of all cancers occur in children, and as a result, patient numbers and funding for dedicated trials are often limited [2]. Consequently, most cancer drug development is focused on adult malignancies, and paediatric oncologists often need to resort to off-label use of therapeutics. *Ad hoc* administration of unapproved (in children) drugs undermines the ethical and systematic development of safe therapeutics for children with cancer and perpetuates the challenges explained previously. To help prioritise mechanism-of-action-based drugs for paediatric clinical development, a systematic literature review strategy was developed to summarise current knowledge into proof-of-concept (PoC) terms as part of the Innovative Therapies for Children with Cancer Paediatric Preclinical Proof-of-Concept Platform (ITCC-P4; Grant Agreement No 116064) [3,4]. Using this structured and stepwise

workflow, our current target actionability review (TAR) evaluates the PoC preclinical data of published literature focused on targeting replication stress in 16 different paediatric intracranial and extracranial solid tumour types.

Replication stress is a cell state that is initiated when the DNA replication fork is stalled during the cell cycle [5]. A variety of mechanisms can contribute to the slowing or stalling of the replisome, but regardless of the reason, delay can result in the accumulation of DNA damage, initiation of genome instability and ultimately cell death [6]. Given that genomic instability and DNA damage are characteristics common to cancer cells, targeting replication stress to exacerbate these effects and induce cell death is a promising therapeutic approach [7].

One strategy to exploit replication stress to kill tumour cells is using classic chemotherapeutics, such as DNA alkylating agents, topoisomerase inhibitors and radiotherapy. These approaches, which are widely used in the clinic, interrupt normal DNA replication and induce toxic accumulation of replication stress [8]. However, the mechanism of these therapies relies on a

high cell proliferation rate that is not limited to malignant cells alone. Using conventional chemotherapies, cells of the gut epithelium and bone marrow are also affected, causing undesirable and intolerable side-effects which often halt treatment and can cause long-term health consequences for children who survive cancer [9].

An alternative, more targeted approach to cause a toxic accumulation of replication stress in cancer cells is to prevent its resolution. When a replication fork is stalled, a network of proteins is activated, which functions to resolve the damage and restart DNA replication [10]. Due to the frequent loss of cell cycle control and/or overexpression/constitutive activation of oncogenes, some cancer cells exist in a perpetual state of replication stress and are thus highly reliant on replication stress response (RSR) pathways for survival [11,12]. Targeted inhibition of proteins within these stress response pathways could be particularly lethal to cancer cells and less toxic to healthy cells and is a novel therapeutic approach with a potentially wider therapeutic window that is being widely studied for the treatment of paediatric and adult cancers.

In this TAR, we gathered and systematically evaluated published preclinical literature focused on targeting the RSR in intracranial and extracranial solid paediatric malignancies. Ultimately, we have highlighted the strengths and gaps in knowledge for multiple drug targets, and with this, we aim to guide further preclinical research and development of novel treatments targeting replication stress in children with cancer.

2. Methods

The TAR methodology we established – which is outlined in Fig. 1 – consisted of four major steps with minor deviations from the original appraisal approach established by Schubert et al. (2020) [3]. Using specific and general keywords, literature related to replication stress in 16 different types of paediatric solid tumours (Table 1) was collected (step 1). If individual articles addressed one of the defined PoC modules (Supplementary Table 2), it was included for further review. In step 2, each article was scored by two separate reviewers using the publicly available web portal R2 (https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?option=imi2_targetmap_v1). Next, the key target addressed in the article was identified, any discrepant scores were adjudicated using an independent third reviewer (step 3), and final scores were assigned and visualised (step 4).

2.1. Step 1: literature search

Using the R package RISmed version 2.2, literature related to replication stress was collected via PubMed queries using replication stress keyword(s) combined with the paediatric tumour type of interest ([‘*pediatric*

tumor’ AND ‘*replication stress keyword*’]; see Table 1 for search term overview and Supplementary Table 1 for detailed search queries) [13]. Literature that were published between 2014 and 2021 (last literature search: 27-01-2021) and contained the search terms in the title and/or abstract were included. Next, abstracts were analysed to determine if they should be included for critical review and scoring. Only literature that addressed one of the PoC modules outlined in Supplementary Table 2, in a paediatric entity listed in Table 1 were included and all review articles were excluded. The final list of PubMed IDs (PMIDs) was uploaded into the R2 TAR platform where they were scored in step 2 of the TAR methodology.

2.2. Step 2: critical review and scoring

Using the previously established critical appraisal PoC modules, each article was evaluated by two separate reviewers and all relevant data were entered as separate evidence entries in the R2 TAR platform [3]. First, the key findings of each article were briefly summarised by both reviewers 1 and 2 according to the guidelines presented in Supplementary Table 2. Any studies evaluating micro- or long non-coding RNA, natural compounds or monotherapy with classical chemotherapy or radiotherapy were excluded. Then, using the scoring criteria outlined by Schubert et al. [3] (2020), each module for each tumour type was assessed, and quality and outcome scores were assigned. The experimental quality scores – reflecting the robustness of the reported findings – ranged from 1 to 3 (Supplementary Table 3). The experimental outcome scores ranged from -3 to +3 and give an indication as to whether the study results warrant the targeting of a specific protein/pathway for the treatment of a paediatric solid or brain tumour (Supplementary Table 4). Once the summary and appraisal scores from both reviewers were entered in the R2 TAR platform, each article proceeded to the adjudication stage.

2.3. Step 3: reviewer adjudication

In the first step of the adjudication process, the collected evidence for each article was re-evaluated by reviewers 1 and 2 together. The main target of each article was identified, and the addressed PoC modules and assigned quality and experimental scores were evaluated. Each module or score that was discrepant between the two reviewers was briefly discussed and adjusted if necessary. Articles with remaining discordant scores were sent to a third independent reviewer who then scored the article while being blind to the original modules and scores are given by reviewers 1 and 2. If the score given by the third reviewer was discordant with the scores given by the first reviewers, the article then entered a second adjudication phase

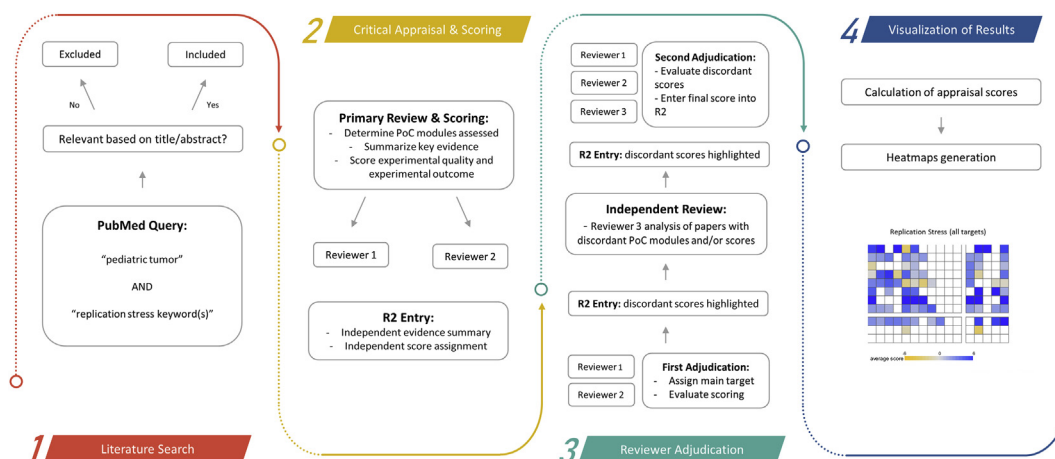


Fig. 1. Overview of target actionability review (TAR) process.

Table 1

Keywords and tumour entities included in PubMed search queries.

Replication stress keywords		Tumour entities
General keywords:	Replication stress	Neuroblastoma (NBL)
	Genomic instability	Rhabdomyosarcoma (RMS)
Specific keywords:	Chromothripsis	Synovial sarcoma (SS)
	BRCA	Malignant peripheral nerve sheath tumour (MPNST)
	R-loops	Ewing's sarcoma (ES)
	Mutational signature	Osteosarcoma (OS)
	MYC amplification	Atypical teratoid/rhabdoid tumour (AT/RT) & Malignant rhabdoid tumour (MRT)
	MYCN amplification	Wilms tumours/nephroblastoma (WT)
	high MYC expression	Hepatoblastoma (HB)
	High MYCN expression	Inflammatory myofibroblastic tumour (IMT)
	Mitotic catastrophe	Retinoblastoma (RB)
	Reactive oxygen species	Extracranial germ cell tumour (extracranial GCT)
	Synthetic lethal treatment	Low-grade glioma (WHO grades I & II; LGG)
		High-grade glioma (WHO grades III & IV, incl. glioblastoma; HGG)
		Ependymoma (EPN)
		Medulloblastoma (MB)
	ATM	
	ATR	
	DNA-PK/DNA-PKcs/PRKDC	
	CHK1/CHEK1	
	WEE1	
	PARP	

where discrepant scores were discussed by reviewers 1, 2 and 3 together, and a single final consensus score was assigned to the article.

2.4. Step 4: visualisation of results

Once final experimental quality and outcome scores were entered in the R2 TAR platform, they were multiplied to create a single appraisal score for each data entry. The final scores ranged from -9 to +9, creating a gradient indicative

of the importance of the study. Finally, the scores for each PoC module within each of the 16 tumour types were averaged to create a heatmap of results. The interactive heatmap can be accessed using the publicly available R2 TAR platform [https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?option=imi2_targetmap_v1] where readers can view the number of articles and average appraisal score for each module in each malignancy type for replication stress overall, as well as per specific target included in our study. In addition, the summarised evidence, individual

scores and PubMed links can be viewed for each entry by clicking on a tile in the heatmap.

3. Results

In this TAR, we systematically evaluated all literature (published between 2014 and 2021) related to targeting replication stress in 16 different paediatric solid tumours. Using the search terms and strategy outlined in the Methods, we gathered 708 unique articles. Next, the literature was curated based on the presence of at least one PoC module in the title/abstract, and 319 articles (45%) entered the critical appraisal stage. In this step, 174 articles did not fulfil the inclusion criteria and were removed from the study (most commonly because of the use of micro or long non-coding RNA, natural compounds or monotherapy with classical chemotherapy/radiotherapy). Ultimately, 145 articles were scored and summarised in 392 evidence entries in the R2 portal. In the first adjudication step, 68 articles (47%) had at least one discrepancy in any of the modules scored and/or the experimental quality/outcome scores assigned. These articles entered the independent review process, and after being scored by a third independent reviewer, 58 discrepancies remained out of the 319 evidence entries (18%). In the second adjudication step, all reviewers discussed and resolved all remaining discordant scores. Finally, appraisal scores were calculated, and publicly available interactive heatmaps were generated for replication stress overall and each of the six main targets included in our study (Fig. 2).

Pervious TARs have focused on single targets (for example MDM2 [3]), thereby naturally limiting the scope of the literature under review. In contrast, replication stress is an extremely broad process involving multiple potential targets, which challenges the established TAR methodology. To address this, we have employed a two-pronged search strategy involving both general and specific replication stress search keywords.

Using the specific keywords, we focus this TAR on six druggable targets involved in replication stress (1) ATM, (2) ATR, (3) CHK1, (4) DNA-PK, (5) PARP and (6) WEE1. These proteins are involved in DNA repair pathways and cell cycle control which are two well-studied components of the RSR (Fig. 3a). Nevertheless,

replication stress extends beyond these targets, and to extract literature on potential targets outside of DNA repair pathways and cell cycle control, we included general search keywords in our search strategy. Altogether, our TAR included 31 alternative replication stress targets (Fig. 3b), which accounted for 127 (32%) of the total evidence entries across all PoC modules. While we were unable to systematically evaluate each alternative target in the scope of this study, the general keyword search of our approach aids in creating an extensive overview of targeting replication stress that can be explored using the interactive heatmap on the R2 TAR Platform [https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?option=imi2_targetmap_v1].

Assessing replication stress overall (which includes both specific targets and alternative targets identified via our general replication stress search words; Fig. 5a), NBL, ES, OS and MB were the most robustly represented malignancies in our study. In addition to having the greatest number of evidence entries (NBL = 79, ES = 71, OS = 71 and MB = 58), all PoC modules were addressed in these malignancy types, creating a more comprehensive overview of targeting replication stress in these tumour types than other tumour types, which had fewer evidence entries (or none in the case of IMT and extracranial GCT). However, despite all PoC modules being represented in NBL, ES, OS and MB, it is important to note that some modules are represented by only one publication. For example, the module ‘target/pathway activation’ in ES received a negative overall appraisal score but is only represented by a single publication. Taken together, these results are not a negative indication for targeting replication stress in ES. Instead, the paucity of data (which was not limited to only this one instance) is indicative of where further preclinical research should be focused. In our TAR overall, we observed underrepresentation of data exploring the biological underpinnings of targeting replication stress (i.e. PoC modules ‘target/pathway activation’, ‘predictive biomarkers’, ‘resistance’) across all tumour types, highlighting the direction for future studies (Fig. 4a).

To further refine the status quo of targeting replication stress for the treatment of paediatric cancer, our TAR also summarises the preclinical data available for

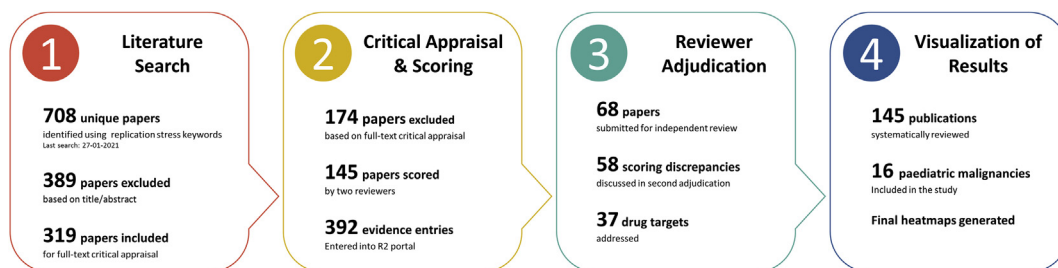


Fig. 2. Literature selection and key evaluation steps throughout TAR workflow. TAR, target actionability review.

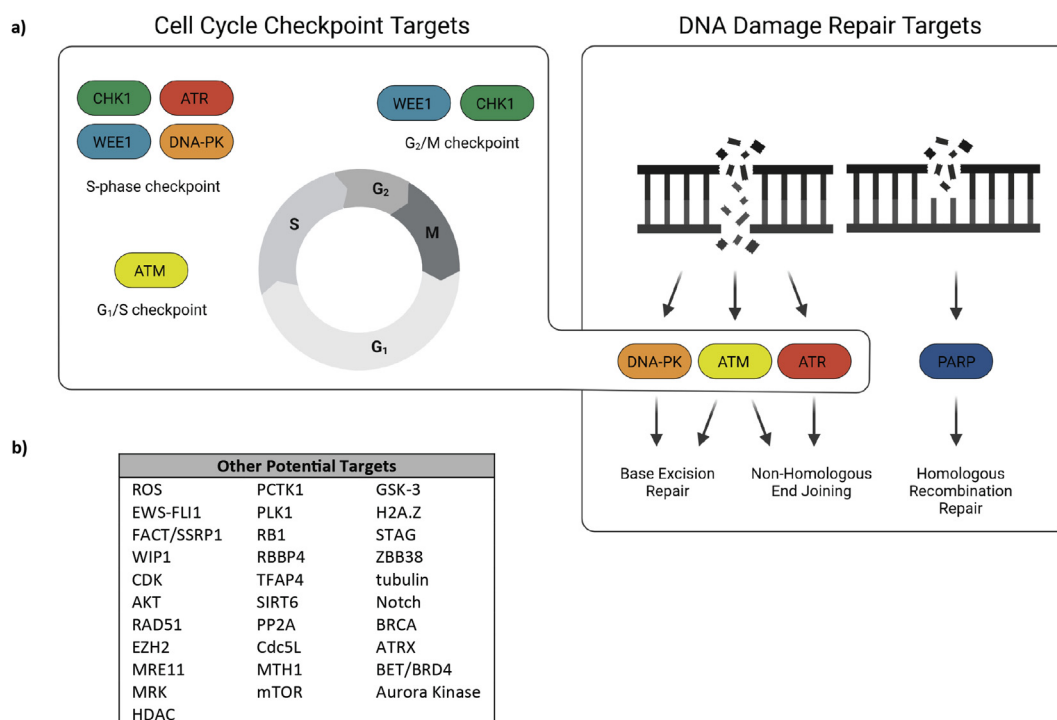


Fig. 3. (a) Overview of cell cycle checkpoint and DNA damage repair targets included specific replication stress keywords. (b) Potential alternative replication stress targets are identified by way of general replication stress keywords.

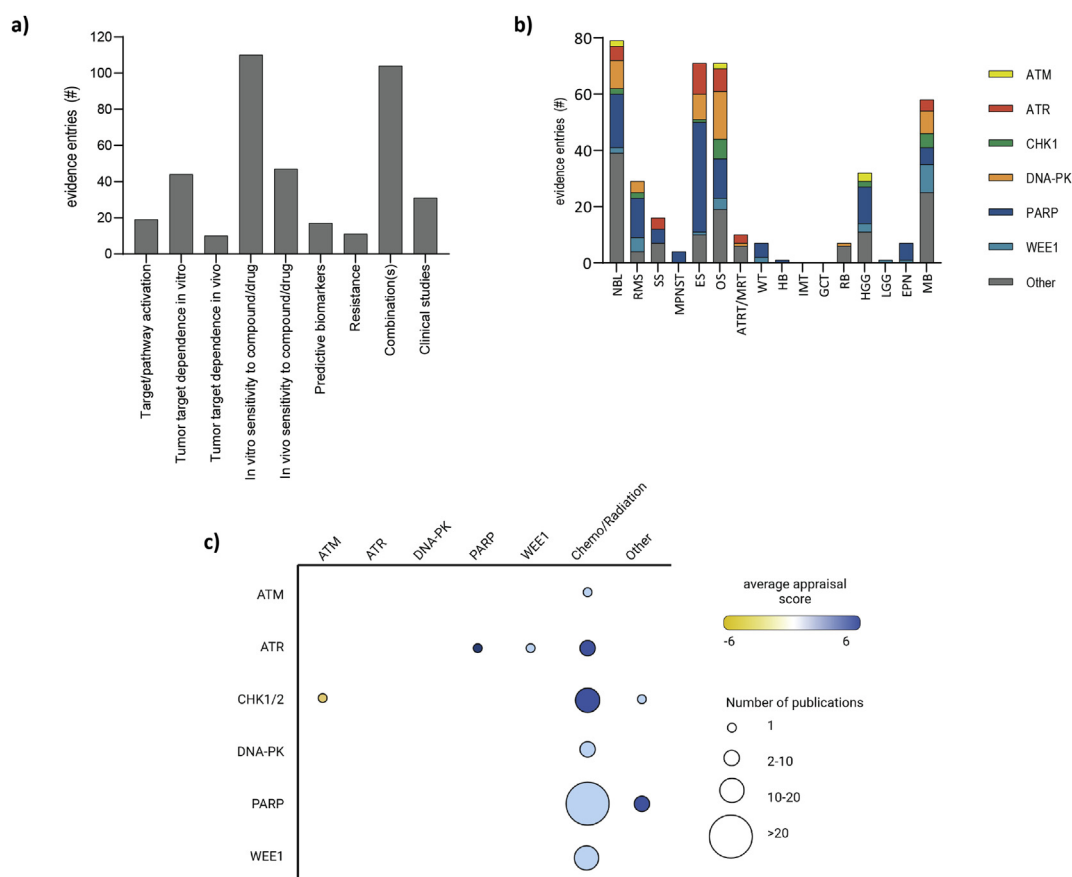


Fig. 4. (a) Number of evidence entries per PoC module entered into the R2 TAR platform. (b) The number of evidence entries per paediatric entity is divided across the different targets of interest. (c) Overview of therapeutic combinations reviewed where the size of the dot represents the number of evidence entries, and the colour represents the average appraisal score for all publications for that combination. PoC, proof-of-concept; TAR, target actionability review.

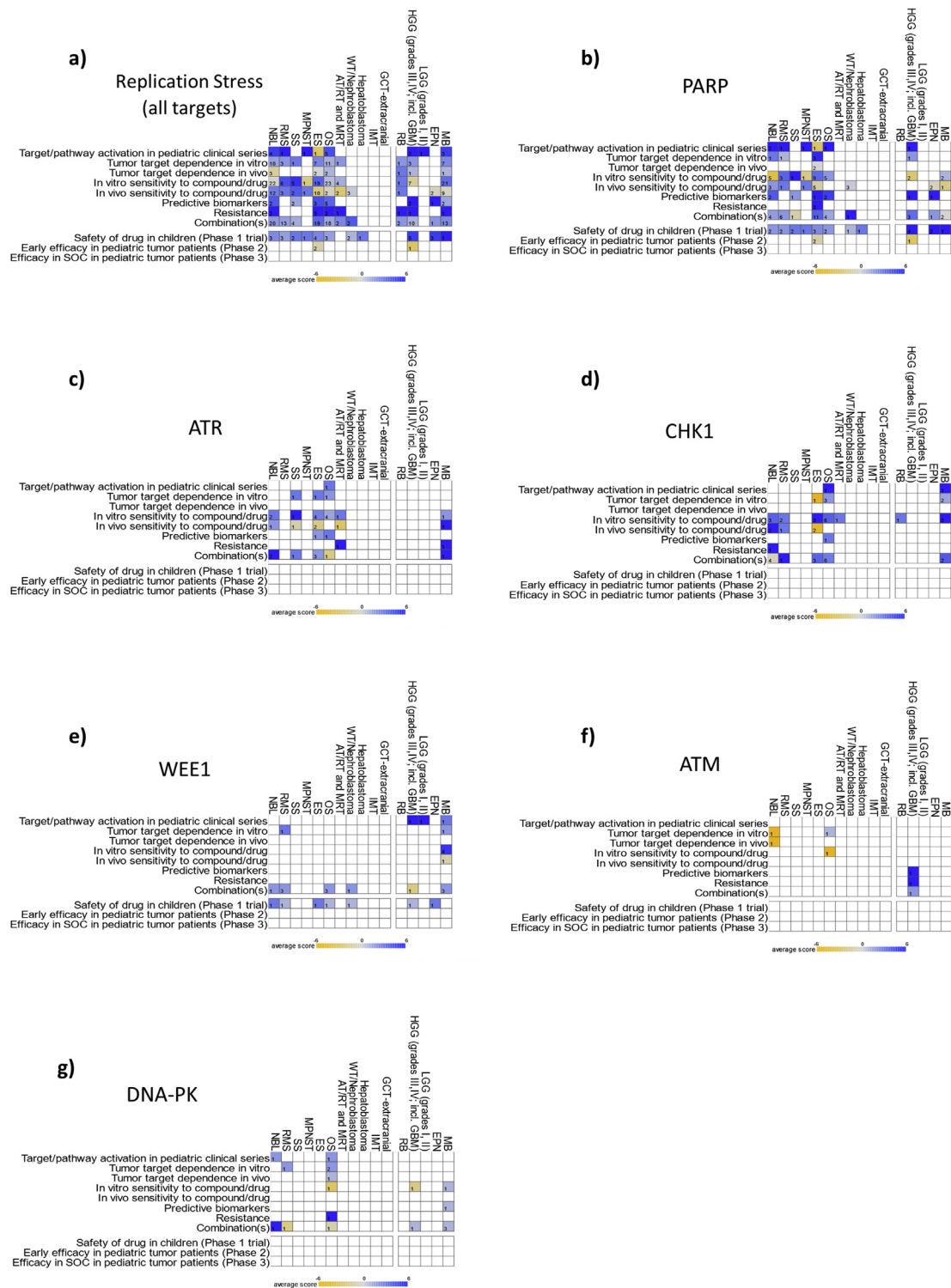


Fig. 5. Summary of appraisal scores for (a) replication stress overall, (b) PARP, (c) ATR, (d) CHK1, (e) WEE1, (f) ATM and (g) DNA-PK. Each box represents the averaged appraisal score in which yellow indicates a negative result and blue indicates a positive result, and the number within indicates the volume of evidence entries. The interactive heatmaps can be accessed via the R2 TAR platform [https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?option=imi2_targetmap_v1].

ATM, ATR, CHK1, DNA-PK, PARP and WEE1 (interactive heatmaps for each target are available on the R2 TAR Platform [https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?option=imi2_targetmap_v1]). Of the specific targets, PARP was the best represented as it accounted for 127 (32%) of the total evidence entries and was the only specific target to cover all nine PoC modules (in all entities except AT/RT & MRT, IMT, GCT, RB and LGG; Fig. 4b). With the volume and robustness of data available for PARP, this target is perhaps the most promising – especially considering the data supporting PARP inhibitor combinations with classic chemotherapeutics (Figs. 4c and 5b). However, that is not to say that the remaining specific targets do not show potential. Though represented by fewer evidence entries overall, ATM ($n = 7$), ATR ($n = 35$), CHK1 ($n = 50$), DNA-PK ($n = 18$) and WEE1 ($n = 29$) remain targets of interest and our TAR functions to define the current landscape of targeting these proteins in paediatric tumours with the hope to guide future research on these targets.

Like PARP, inhibitors of all other specific targets in our study were also investigated in combination with classic chemotherapy. Interestingly, CHK1 and ATR both received higher average appraisal scores in the ‘combinations’ module than PARP, suggesting great potential in these therapeutic approaches (Fig. 4c). Moreover, both ATR and CHK1 scored highly overall modules in MB (Fig. 5c and d). Although based on only one article, MB scored positively in all addressed modules for ATR (*in vitro/in vivo* sensitivity to compound’, ‘resistance’ and ‘combinations’), suggesting that this target might be of particular interest in MB [14]. In addition to ATR, MB also scored highly in all modules assessed for the target CHK1 (‘target/pathway activation’, ‘tumour target dependence *in vitro*’, ‘*in vitro*

sensitivity to compound’ and ‘combinations’). Interestingly, CHK1 generally scored positively across all included tumour types (Fig. 6). An exception worth mentioning is ES, which scored negatively in the modules ‘tumour target dependence *in vitro*’ and ‘*in vivo* sensitivity to compound’, contradicting the positive results for ES in the modules ‘*in vitro* sensitivity to compound’ and ‘combinations’ (Fig. 5d). Given that these scores are derived from a limited number of publications (2–3 for each module addressed in ES), further studies should be conducted to fairly evaluate CHK1 as a target in ES. Another noteworthy target for its overall high scores is WEE1. While based on limited literature, WEE1 received overall positive scores across all malignancies (Fig. 6). In addition, combination treatment with a WEE1 inhibitor and chemotherapeutic agent resulted in better responses than WEE1 inhibition alone (Fig. 5e). Moreover, a Phase I clinical trial using WEE1 inhibition in combination with irinotecan scored positively in NBL, HGG, EPN, RMS, ES, OS and WT [15]. The lack of data across the other PoC modules – particularly ‘predictive biomarkers’ and ‘resistance’ (which were not addressed at all) – strongly suggests that additional research should be conducted to further explore this target in paediatric tumour types.

The remaining targets (ATM and DNA-PK) were the least encountered specific targets in our study and were often represented by only one publication across a limited number of tumour types and PoC modules. For instance, ATM included seven evidence entries that only addressed a few PoC modules in NBL, OS and HGG (Fig. 5f). Despite the limited data, HGG scored positively overall, suggesting the efficacy of targeting ATM in this tumour type and demanding further research (Fig. 6) [16,17]. DNA-PK, on the other hand, was more diversely represented than ATM as it addressed all

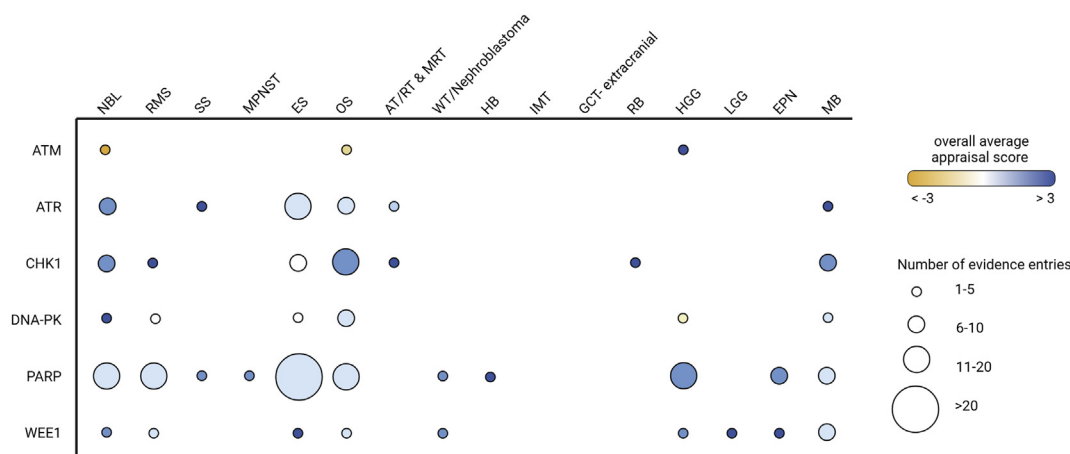


Fig. 6. Overview of specific therapeutic targets across all paediatric entities. The colour of the dot represents overall average appraisal scores derived from all PoC modules for that target and malignancy, and the size of the dot represents the number of evidence entries. PoC, proof-of-concept.

preclinical PoC modules except for ‘*in vivo* tumour target dependence’ across 5/16 of the included tumour types (NBL, RMS, OS, HGG and MB; Fig. 5g). Results for ‘resistance’ in OS [18] and ‘combinations’ in NBL [19] were the highest scoring modules for DNA-PK suggesting that combination treatment with DNA-PK inhibitors could be an effective strategy in certain tumours; however, DNA-PK scored relatively neutrally across all modules and tumour types, underscoring the need for further preclinical research to fairly evaluate this target in paediatric tumours (Fig. 6).

4. Discussion

The TAR methodology aims to match mechanism-of-action-based anti-cancer drugs with specific cancer subtypes based on preclinical studies. In our study, we took the TAR methodology one step further to systematically evaluate the broader ‘target’ of replication stress, which encompasses numerous targetable proteins. To create more resolution in our study, we focused on six specific drug targets to gain insights into which targets within replication stress should be prioritised for preclinical development. Unsurprisingly, this presented unique challenges to the TAR methodology. As summarised in Fig. 6, each of the specific targets in our study were represented by different amounts of literature and overall average appraisal scores varied per target, depending on which malignancy was being investigated. These factors made it exceedingly difficult to define which specific targets warrant priority in future studies. Furthermore, by investigating a target as diverse as replication stress, it was required that our search strategy was well-defined and stringent, which defies the very nature of the topic at hand. Together these represent some of the unique limitations that were observed in addition to some of the broader constraints inherent to the TAR methodology overall. For example, it is not possible to account for the highly dynamic and sometimes unexpected patient responses in clinical trials, and because of the rarity of some tumour types, it is impossible to create an equal summary of evidence across all tumour entities.

Despite these challenges, replication stress remains an attractive therapeutic approach, and the broad nature of the RSR offers a multitude of novel therapeutic approaches, which we have explored in this TAR. In our study, we have systematically reviewed replication stress as a therapeutic target while focussing on six specific drug targets, ATM, ATR, CHK1, DNA-PK PARP and WEE1. Of these six targets, PARP emerged as the most widely studied and promising therapeutic target.

In addition to addressing all PoC modules and being represented by the greatest amount of literature, PARP also scored positively across all investigated tumour entities. Of specific interest are the results of the

‘combinations’ module where PARP inhibitors combine synergistically with classic chemotherapeutics. Two-fold targeting of replication stress in this manner could harnesses the power of chemotherapy while limiting the toxic side-effects associated with high-dose treatment. The results of our study support this notion, for instance, in the ‘phase I clinical studies’ module where PARP was combined with chemotherapy (temozolomide and/or irinotecan) or radiotherapy [20–24]. Across the different malignancy types, overall appraisal scores were positive, which suggests that combination treatment with PARP inhibitors and classic chemotherapy/radiotherapy is generally well tolerated by patients. However, the results of ‘phase II clinical studies’ – a module that is notable for its marked absence of data across all potential replication stress targets – do not demonstrate remarkable clinical efficacy. Based on the limited data available, results varied from no clinical benefit to stable disease for HGG [20] and ES [24,25], respectively, highlighting the need for further investigation.

Interestingly, this two-fold approach to targeting replication stress was not solely observed in literature focused on PARP. The ‘combinations’ module was addressed in all other targets included in our study, and most of the other studies also combined targeted inhibitors with classic chemotherapeutics. Given the role these proteins play in DNA damage repair pathways, it is unsurprising that most demonstrated minor synergy when combined with chemotherapy or radiation. However, most prominent were the results of CHK1 inhibition combined with chemotherapy which consistently demonstrated synergy in MB, ES, OS, NBL and RMS [26–28]. In addition, there were some studies that investigated combination treatment with two RSR-targeted inhibitors (such as ATR with WEE1 [29] or PARP [30] inhibitors). Dual targeting of replication stress without the use of chemotherapy is also an appealing therapeutic approach as it does not rely on therapy which is known to induce toxicity, which can have long-term health consequences [9]. Furthermore, studies combining RAF and MEK inhibitors in metastatic melanoma demonstrate that targeting multiple proteins within the same signalling pathway is an effective way to achieve greater therapeutic effects in adults and prompts the question of whether this is also a valid approach in targeting replication stress in paediatric tumours [31]. In our TAR, two different targeted strategies were observed: (1) a ‘vertical blockade’ in which two proteins within the same signalling axis were inhibited and (2) a ‘lateral blockade’ where two proteins involved in different signalling pathways within the RSR were inhibited. While these two strategies are scarcely represented in our study, ‘lateral blockade’ with ATR and PARP inhibitors in NBL [30] and ‘vertical blockade’ with ATR and WEE1 inhibitors in ES [29] both demonstrated synergy. Considering the success of

‘vertical blockade’ strategies in adult malignancies, we think targeting replication stress with a similar strategy could offer better therapeutic efficacy with fewer side-effects in paediatric tumours than combination strategies, including chemotherapeutics/radiation; however, more preclinical research needs to be generated across all targets and paediatric tumour types to determine which targets and strategy are superior.

In addition to intelligent combination strategies, improving treatment efficacy also hinges on robust preclinical studies. With a thorough understanding of the biological mechanisms driving a treatment approach, models/patients with the appropriate molecular background can be selected to ensure a fair evaluation of the therapy. In our study overall, we observe a glaring underrepresentation of literature focused on unravelling the biological underpinnings of targeting replication stress for therapeutic purposes. Across all targets and tumour types, ‘predictive biomarkers’ and ‘resistance’ were two of the poorest represented modules and together accounted for less than 10% of all evidence entries. Furthermore, we noted that tumour subtypes were often not reported, and some tumour types were not represented at all (i.e. IMT and extracranial GCT). While these underrepresentations can partially be attributed to the way research is prioritised and the availability of preclinical models, a more robust representation across tumour (sub)types could provide useful insights into treatment stratification and should be addressed in the future studies. For example, the EWS-FLI1 fusion gene (observed in ~85% of ES patients) has been implicated in deficient DNA repair mechanisms, inefficient DNA transcription and overall increases in replication stress [32,33]. In the context of therapeutic targeting of replication stress, this could be a very important subtype of ES to consider. Understanding treatment mechanisms and the effects that different tumour (sub)types might have on their functioning is crucial to designing successful clinical trials, and the paucity of literature addressing these topics in our TAR presents a potential gap in our knowledge.

5. Conclusion

Overall, we have used the TAR methodology to create a comprehensive, structured and critically evaluated overview of literature related to targeting replication stress in paediatric solid malignancies, which can be easily explored via the publicly available interactive heatmap on the R2 TAR Platform [https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?option=imi2_targetmap_v1]. By summarising our results according to specific targets, we observe a large representation of literature supporting the further development of PARP inhibitors in certain paediatric tumour entities.

Furthermore, our results highlight emerging targets such as CHK1 and WEE1 and demonstrate the potential of novel combination strategies that target the RSR. Finally, we underscore the importance of robust preclinical studies and intelligent clinical trial design and highlight where further research is necessary with the hope of helping the development of safe and effective therapeutics for children with cancer.

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Conflict of interest statement

Louis F. Stancato is full-time employee of Eli Lilly and Company. Ana Rodríguez and Hubert N. Caron are fulltime employees of Hoffmann-La Roche. All remaining authors have declared no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejca.2021.11.030>.

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