Supplementary 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	***Is the target pathway active in the tumour of interest?***	Total size of cohort (consider only the number of patient samples, not cell lines)
	Target/pathway evaluation in clinical series: DNA aberrations, (over)expression, methylation changes?	Methodology used
	Target DNA aberrations: Mutation, translocation, amplification, in/del, CNV	Percent of samples expressing the target (and associated alterations or mutation) or with activated target pathway
	Percent of samples with aberrant target/pathway in clinical series	
	Distribution over clinical risk groups	
	Correlation to clinical outcome	
	Correlation to other tumour biology	
	Target expression/pathway activity compared to normal tissue, other cancers, and/or other reference tissue	
Tumour target dependence **PoC 2: I*n vitro***	***Is the tumour of interest dependent on the target or pathway for survival?*** ***In vitro***	***In vitro/in vivo***
	Molecular target gene silencing in cells (RNAi, AOs, CRISPR, etc.) or ectopic expression; preferably≥ 3 cell lines	Model(s)
	Phenotype analysis (apoptosis, cell viability, etc.)	Methodology used
	Biological effect of molecular silencing or ectopic expression of target Appropriate controls (use of multiple silencing tools, rescue experiments, control cell lines, etc.) Additional functional assays showing target or pathway dependence for mutated/translocated/amplified target genes	Results of initial experiment (generally, cell viability or tumour growth) Rescue experiment used Validation (effects on apoptosis, proliferation, cell cycle, migration, gene or protein expression, etc.)
PoC 3: *In vivo*	***In vivo***	
	Molecular silencing or overexpression of target gene in xenografts (inducible shRNA or expression vectors) Transgenic models (mice, zebrafish, etc.) for mutated/translocated/amplified target genes or for activated pathways	
Sensitivity to tool compound/drug	***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)?)***	
PoC 4: *in vitro*	***In vitro***	***In vitro***
	Preferably ≥4 cell lines with target dependence (preferably with ≥1 control cell line without target dependence)	Type (established cell line or patient-derived [i.e. *ex vivo*]) and number of cell lines used [including controls]) Drug(s) used and concentration range tested; time point(s) used to assess cell viability Percent of sensitive lines (IC_{50} ≤ 500 nM or clinically relevant [if known/applicable]) Validation (effects on apoptosis, proliferation, cell cycle, migration, gene or protein expression, etc.)
readout 1	Cell viability: IC_{50}, GI_{50}, LC_{50}, survival curves	
readout 2	Biological efficacy: Preferably measured with pharmacodynamic (PD) assays intended for extrapolation to clinical studies	
	Correlation of efficacy with tumour biology	
PoC 5: *in vivo*	***In vivo***	***In vivo***
	Xenografts / PDX / GEMM (both with dependency on evaluated target) Preferably measured with predictive biomarker to be used in clinical trial for patient selection	Model(s) (cell-line or patient-derived xenografts, transgenic mice, orthotopic v subcutaneous, etc.) and *n*/arm Dosing schedule used
readout 1	Pharmacokinetics (PK; plasma and intratumoural)	Tumour growth inhibition and/or overall response extrapolation for each experiment
readout 2	Pharmacodynamics in tumour: 1. target binding, 2. target inhibition, 3. pathway modulation, 4. biological effect PK - PD relationships: Preferably use assays intended for extrapolation to clinical studies	Validation (effects on apoptosis, proliferation, cell cycle, migration, gene or protein expression, etc.)
readout 3	Response rates and survival measures (use established, measurable tumours)	
	Efficacy - PD - PK relationships	
PoC 6: predictive biomarkers	***Can biological compound efficacy be determined by a specific marker in preclinical models?***	Biomarker(s) reported
	Evaluation of existing, validated biomarkers in PoC4 and PoC5	*In vitro/in vivo* correlation (include statistical values if available)
	Predictive biomarker (intended for extrapolation to clinical studies and patient selection)	Patient correlation (include statistical values if available)
	Efficacy biomarkers (PD markers)	Patient correlation (include statistical values if available)
PoC 7: resistance	***Are the mechanisms of resistance understood?***	Model(s) (*in vitro/in vivo*)

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