ITCC P4 PAEDIATRIC PRECLINICAL PROOF OF CONCEPT PLATFORM

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Target Actionability in Pediatric Cancers

September 27th 2018

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ITCC-P^4

Pediatric Target Actionability

Clinical Compound → **Mechanism of Action**

Pediatric Tumor Biology → **Target Actionability**

Match

Target classes:

- Tumor dependance
  - ✓ Genomic
  - ✓ Expression (lineage)
- Tumor delivery
- Tumor microenvironment
  - ✓ Immunology
  - ✓ Angiogenesis

FDA Target Classes:

- *Gene Abnormality Targets*
- *Cell Lineage Targets*
- *Non-cancer cell Targets*
- *Other Targets*

Is the target actionable:

(in tumor specific context)

- Is the target (pathway) activated in tumor of interest?
- Is tumor survival dependent on the target?
- Can a matched drug kill the tumor in relevant models?
- How does resistance occur?
- Are combinations more effective?

Pediatric Therapeutic Potential

Outline

- Defining POC datapackages
  - ✓SIOP Target Actionability Taskforce
  - ✓POC categorization
  - ✓POC data wishlist
- POC data reviews (‘Target Actionability Reviews’)
- Minimally required POC data per disease:
  - ✓Clinico-biological disease sub-categories defined by the disease experts
  - ✓NB pilot for definition of ‘minimal POC package’
  - ✓PoC data package flowchart

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SIOP Target Actionability Taskforce, 2014

Defining Proof-of-Concept datasets

- ✓60 international pediatric oncology experts
- ✓Preclinical POC dataset definition:
  - Literature search by core team
  - Extraction of preclinical POC data items + POC testing recommendations
  - Design POC data categories based on data types
  - Delphi round with all experts:
    - Refine POC data categories
    - Define ‘wishlist of preclinical POC data types’
  - Workshop (SIOP Toronto 2014):
    - Finalize POC data categories
    - Finalize POC data type listing

Preclinical Proof-of-Concept Data categories

Module 1	Target Activation Status in clinical series
Module 2	Target Dependence: 'in vitro' (molecular validation)
Module 3	Target Dependence: 'in vivo' (molecular validation)
Module 4	Molecule Sensitivity Patterns 'in vitro'
Module 5	Molecule Efficacy 'in vivo'
Module 6	Biomarkers; Predictive and Biological Efficacy (PD) (confirmation)
Module 7	Resistance mechanisms
Module 8	Rational combinations
Clinical data	*Clinical trials*

See Appendix for 'POC wishlist

Outline

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Systematic Literature Reviews of Target Actionability

STEP 0: Expert reviewers

- Identify 2 or more reviewers
- Review methodology and R2 datatool
- Derive specifics for target patterns and target validation from basic target (pathway) biology in cancer

STEP 1: Sensitive literature search for papers on pediatric tumors of interest

- Sensitive PubMed search
- Select relevant papers, based on Title + Abstract
- Obtain full papers

STEP 2: Critical evaluation of papers and scoring of main findings

(independent by each reviewer)

- Critical reading of papers
- Extract Main Finding(s) per paper
- Categorize each main finding for disease entitie(s) and for class(es) of POC data
- Appraise + score main findings for Experimental Quality and for Effect Quantity (standardized guidance tables)

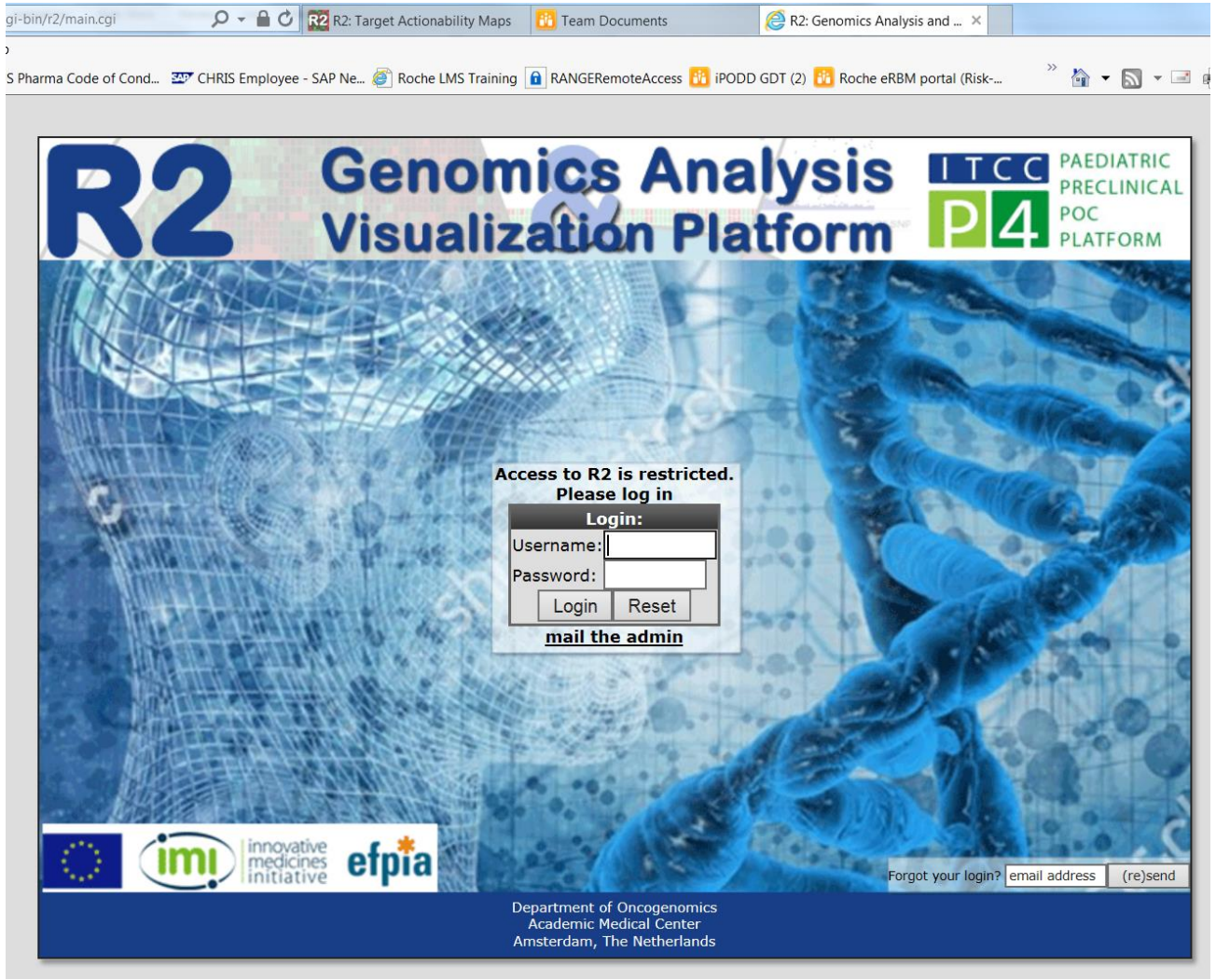
STEP 3: Comparison of the scoring of independent reviewers

- Reviewer discussion of main findings and evidence scores per tumor entity
- Resolve discrepancies by discussion
- Compile 1 adjudicated review table

STEP 4: visualization in R2 datatool

- Upload adjudicated review table in R2
- Derive POC heatmap from evidence scores (colour code = Average of 'Quality * Quantity')

ITCC-P4 WP2:
Pediatric Target Actionability



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https://hgserver2.amc.nl/cgi-bin/r2/main.cgi — R2: Target Actionability Maps — R2: Target Actionability Ma...

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Lifecycle Management Po... | US Pharma Code of Cond... | CHRIS Employee - SAP Ne... | Roche LMS Training | RANGERemoteAccess | iPODD GDT (2) | Roche eRBM portal (Risk-... | Page | Safety | Tools

Go to Main — **R2: Target Actionability Maps** — Hel

- Main
- ITCC-P4 (IMI2)
- Time series
- AmpliconView
- Kaplan Meier
- Tools
- t-SNE Maps
- Genome Browser
- ChIP data
- Help
- My Settings
- Change Species
- Change Data Scope
- Contact / About R2

Enter Reviewer Mode

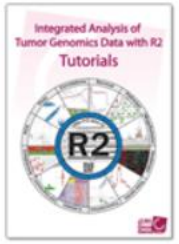
Adjustable settings

Map: MDM2(tp53)_v2a idasanutlin

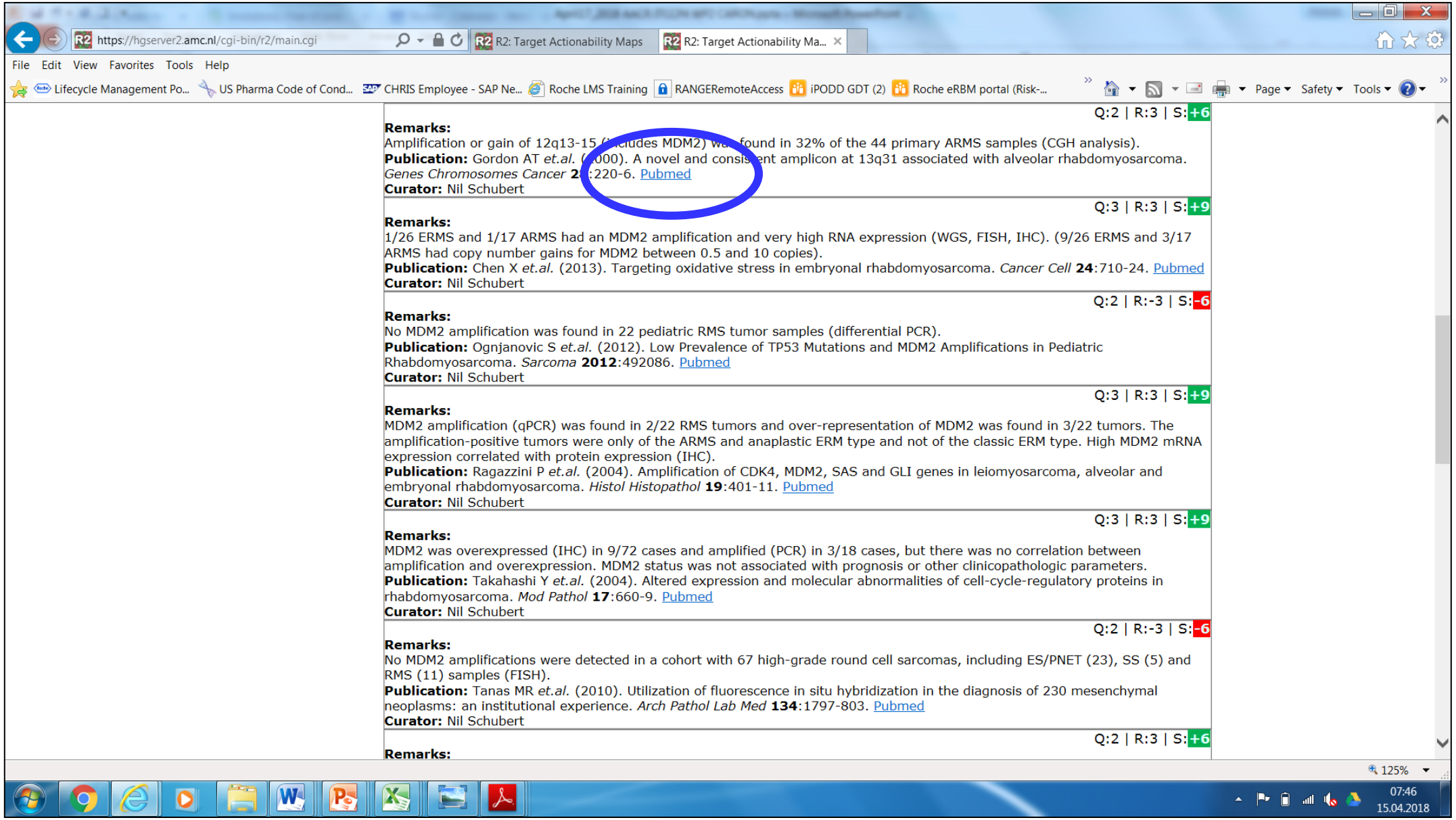
Next | Reset

Target/pathway: **MDM2-TP53**
Version Date: *13 April 2018*
Author: *Nil Schubert, Guillaume Bergthold, Caitlin Lowery*

		Neuroblastoma	Rhabdomyosarcoma	STS non-RMS: Synovial Sarcoma	STS non-RMS: MPNST	Ewing sarcoma	Osteasarcoma	ATRT / Rhabdoid	Wilms tumor (Nephroblastoma)	Hepatoblastoma	GCT extracranial	Retinoblastoma	HGG (High Grade Glioma, incl GBM)	LGG (Astrocytoma gr.I-III)	DIPG	Ependymoma	Medulloblastoma
Preclinical																	
1.Target activiation in pediatric clinical series	p53 activatable																
	MDM2 amplified																
	MDM2 expressed																
2.Tumortarget dependence (*in vitro* models)																	
3.Tumortarget dependence (*in vivo* models)																	
4. Compound sensitivity (*in vitro* models)																	
5. Compound POC Efficacy (*in vivo* models)																	
6. Biomarker (Predictive and PD)																	
7. Resistance Mechanisms																	
8. Combinations																	



Download the R2 Tutorials Book



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Q:2 | R:3 | S:+6

Remarks:
Amplification or gain of 12q13-15 (includes MDM2) was found in 32% of the 44 primary ARMS samples (CGH analysis).
Publication: Gordon AT *et.al.* (2000). A novel and consistent amplicon at 13q31 associated with alveolar rhabdomyosarcoma. *Genes Chromosomes Cancer* **29**:220-6. Pubmed
Curator: Nil Schubert

Q:3 | R:3 | S:+9

Remarks:
1/26 ERMS and 1/17 ARMS had an MDM2 amplification and very high RNA expression (WGS, FISH, IHC). (9/26 ERMS and 3/17 ARMS had copy number gains for MDM2 between 0.5 and 10 copies).
Publication: Chen X *et.al.* (2013). Targeting oxidative stress in embryonal rhabdomyosarcoma. *Cancer Cell* **24**:710-24. Pubmed
Curator: Nil Schubert

Q:2 | R:-3 | S:-6

Remarks:
No MDM2 amplification was found in 22 pediatric RMS tumor samples (differential PCR).
Publication: Ognjanovic S *et.al.* (2012). Low Prevalence of TP53 Mutations and MDM2 Amplifications in Pediatric Rhabdomyosarcoma. *Sarcoma* **2012**:492086. Pubmed
Curator: Nil Schubert

Q:3 | R:3 | S:+9

Remarks:
MDM2 amplification (qPCR) was found in 2/22 RMS tumors and over-representation of MDM2 was found in 3/22 tumors. The amplification-positive tumors were only of the ARMS and anaplastic ERM type and not of the classic ERM type. High MDM2 mRNA expression correlated with protein expression (IHC).
Publication: Ragazzini P *et.al.* (2004). Amplification of CDK4, MDM2, SAS and GLI genes in leiomyosarcoma, alveolar and embryonal rhabdomyosarcoma. *Histol Histopathol* **19**:401-11. Pubmed
Curator: Nil Schubert

Q:3 | R:3 | S:+9

Remarks:
MDM2 was overexpressed (IHC) in 9/72 cases and amplified (PCR) in 3/18 cases, but there was no correlation between amplification and overexpression. MDM2 status was not associated with prognosis or other clinicopathologic parameters.
Publication: Takahashi Y *et.al.* (2004). Altered expression and molecular abnormalities of cell-cycle-regulatory proteins in rhabdomyosarcoma. *Mod Pathol* **17**:660-9. Pubmed
Curator: Nil Schubert

Q:2 | R:-3 | S:-6

Remarks:
No MDM2 amplifications were detected in a cohort with 67 high-grade round cell sarcomas, including ES/PNET (23), SS (5) and RMS (11) samples (FISH).
Publication: Tanas MR *et.al.* (2010). Utilization of fluorescence in situ hybridization in the diagnosis of 230 mesenchymal neoplasms: an institutional experience. *Arch Pathol Lab Med* **134**:1797-803. Pubmed
Curator: Nil Schubert

Q:2 | R:3 | S:+6

Remarks:

125%

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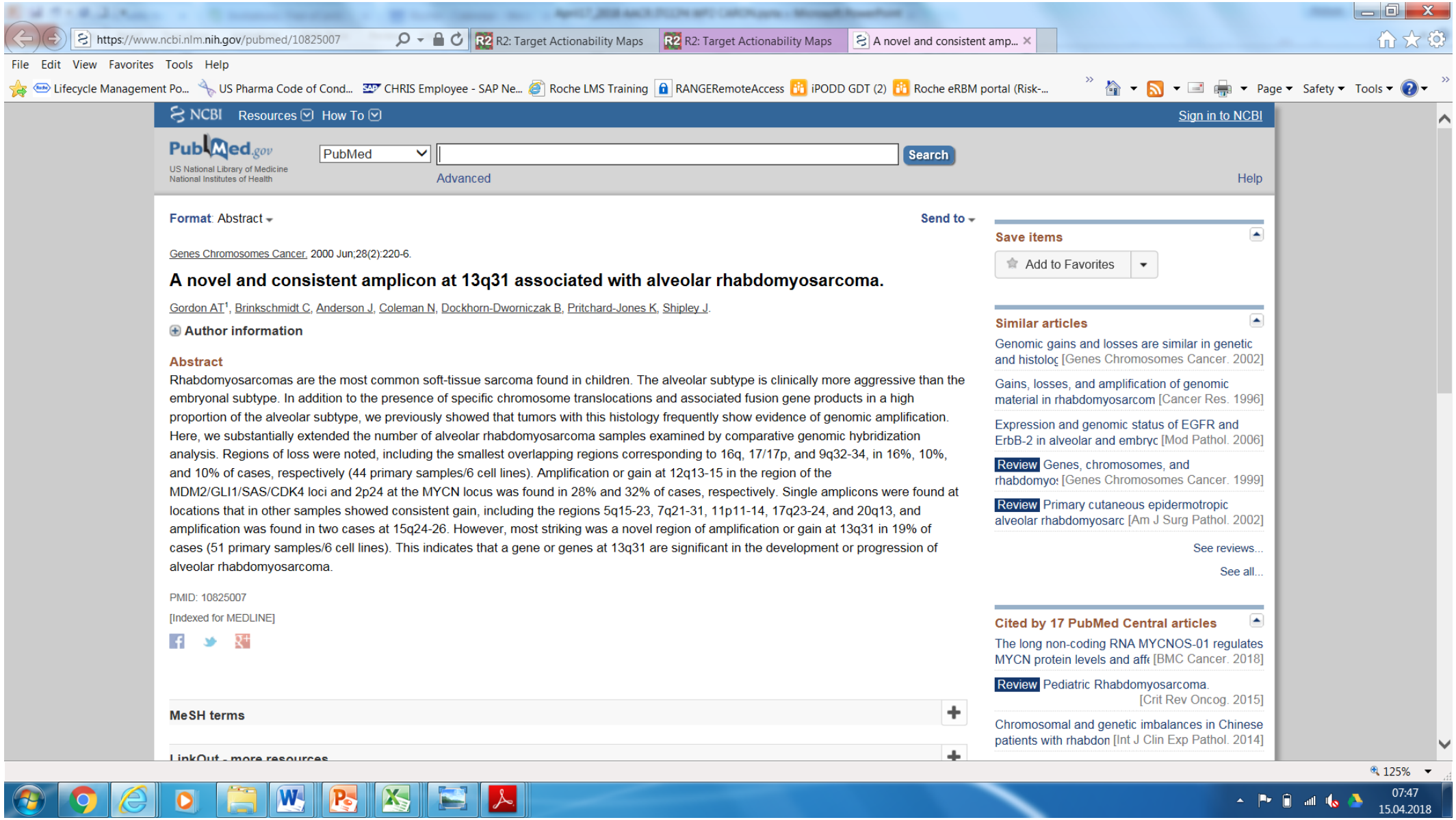
15.04.2018


https://www.ncbi.nlm.nih.gov/pubmed/10825007
R2: Target Actionability Maps
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A novel and consistent amp...
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US National Library of Medicine
National Institutes of Health
PubMed
Search
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Format: Abstract
Send to
Genes Chromosomes Cancer. 2000 Jun;28(2):220-6.
A novel and consistent amplicon at 13q31 associated with alveolar rhabdomyosarcoma.
Gordon AT[1], Brinkschmidt C, Anderson J, Coleman N, Dockhorn-Dworniczak B, Pritchard-Jones K, Shipley J.
Author information
Abstract
Rhabdomyosarcomas are the most common soft-tissue sarcoma found in children. The alveolar subtype is clinically more aggressive than the embryonal subtype. In addition to the presence of specific chromosome translocations and associated fusion gene products in a high proportion of the alveolar subtype, we previously showed that tumors with this histology frequently show evidence of genomic amplification. Here, we substantially extended the number of alveolar rhabdomyosarcoma samples examined by comparative genomic hybridization analysis. Regions of loss were noted, including the smallest overlapping regions corresponding to 16q, 17/17p, and 9q32-34, in 16%, 10%, and 10% of cases, respectively (44 primary samples/6 cell lines). Amplification or gain at 12q13-15 in the region of the MDM2/GLI1/SAS/CDK4 loci and 2p24 at the MYCN locus was found in 28% and 32% of cases, respectively. Single amplicons were found at locations that in other samples showed consistent gain, including the regions 5q15-23, 7q21-31, 11p11-14, 17q23-24, and 20q13, and amplification was found in two cases at 15q24-26. However, most striking was a novel region of amplification or gain at 13q31 in 19% of cases (51 primary samples/6 cell lines). This indicates that a gene or genes at 13q31 are significant in the development or progression of alveolar rhabdomyosarcoma.
PMID: 10825007
[Indexed for MEDLINE]
MeSH terms
LinkOut - more resources
Save items
Add to Favorites
Similar articles
Genomic gains and losses are similar in genetic and histolog [Genes Chromosomes Cancer. 2002]
Gains, losses, and amplification of genomic material in rhabdomyosarcom [Cancer Res. 1996]
Expression and genomic status of EGFR and ErbB-2 in alveolar and embryc [Mod Pathol. 2006]
Review Genes, chromosomes, and rhabdomyo [Genes Chromosomes Cancer. 1999]
Review Primary cutaneous epidermotropic alveolar rhabdomyosarc [Am J Surg Pathol. 2002]
See reviews...
See all...
Cited by 17 PubMed Central articles
The long non-coding RNA MYCNOS-01 regulates MYCN protein levels and affe [BMC Cancer. 2018]
Review Pediatric Rhabdomyosarcoma. [Crit Rev Oncog. 2015]
Chromosomal and genetic imbalances in Chinese patients with rhabdon [Int J Clin Exp Pathol. 2014]
125%
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15.04.2018

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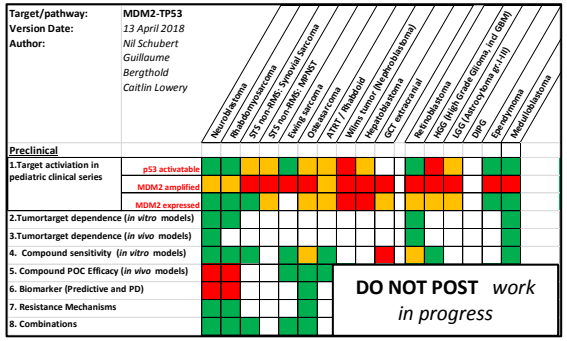
Table 3. Scoring of Evidence Quality

Module	Criteria	Scoring	Criteria
1. Target pattern	number of samples/pediatric patients type of analysis	3	n>20 , two or more different methods
		2	n>10<20, at least one reliable method
		1	n<10, one method
2. Target validation in vitro	knockdown/knockout Confirmation and analysis of knockdown	3	Different methods to induce knockdown/knockout of >3 cell lines + phenotypic analysis of knockdown
		2	Single methods to induce knockdown/knockout of < 3 cell lines
		1	questionable knockdown/knockout
3. Target validation in vivo	type of in vivo model used validation in vivo	3	transgenic mouse model and/or at least 2 different xenografts with an appropriate control and/or different methods of genetic modification in vivo (shRNA /CRISPR) + validation
		2	at least 2 different xenografts without appropriate control + validation
		1	no validation of the developed tumors
4. Drug efficacy in vitro	number of cell lines validation including PD biomarkers or phenotypic response	3	5 cell lines or more + at least two appropriate controls + validation
		2	2-5 cell lines + at least one appropriate controls + validation
		1	1 cell line and/or lack of control +/- validation
5. Drug efficacy in vivo	number and type of in vivo models used	3	2 or more xenograft models or one transgenic mouse model with appropriate control + validation
		2	1 xenograft model with appropriate control + validation
		1	1 xenograft model w/o appropriate control or w/o validation
6. Biomarkers	confirmation of correlation patient selection	3	correlation molecularly confirmed in 2 or more models (e.g. silencing, overexpression, etc.)., patient selection
		2	correlation confirmed in one model, patient selection
		1	correlation not confirmed
7. Resistance	development of resistance molecular analysis overcoming resistance	3	reported resistance + comprehensive analysis + reversing/overcoming resistance
		2	reported resistance + analysis of molecular changes underlying or due to resistance
		1	only reporting resistance
8. Combinations	concentrations tested combination index (CI) in vitro / vivo combination	3	>4 concentrations of each compound are tested + CI + in vivo
		2	1-4 concentrations of each compound are tested + CI +/- in vivo
		1	1 concentration of each compound is tested

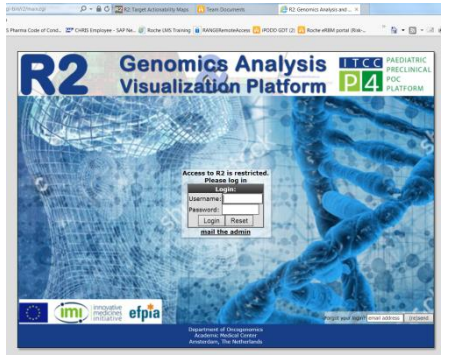
Table 4. Scoring of Evidence Quantity

Module	Criteria	Scoring	
1. Target pattern	Prevalence of abnormal expression/amplification/mutation in cohort (separate scoring)	3	More than 10% in the cohort
		1	Between 2-10%
		-3	No
2. Target validation in vitro	Level of dependency and phenotypic recapitulation	3	Full dependency (>75% cell death OR transformation) after knockdown/knockout
		1	Partial dependency (<75% death OR growth arrest)
		-3	No dependency
3. Target validation in vivo	Level of dependency and phenotypic recapitulation	3	Full dependency (CR / complete tumor regression) after knockdown/knockout or transformation in GEMM
		1	Partial dependency
		-3	No dependency
4. Drug efficacy in vitro	IC50 observed after 72hr exposure	3	< 500 nM
		1	500-1500nM
		-1	>1500 nM
		-3	No activity (> 10uM)
5. Drug efficacy in vivo	In vivo tumor response extrapolation (preferably using clinically relevant dose)	3	Response comparable to PR/CR
		1	Response comparable to SD
		-1	Very minor response (between SD and PD)
		-3	No activity or clear PD, comparable to control
6. Predicitive biomarker	Confirmation of correlation	3	Strong correlation
		1	Moderate correlation
		-3	No correlation
7. Resistance	Reported resistance	3	Resistance reported with drug exposure (at clinically relevant dose) with identification of mechanism of resistance
		1	Resistance reported with no identification
8. Combination	Synergy - CI	3	Strong synergy reported - 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 synergy

ITCC-P4 WP2:
Pediatric Target Actionability

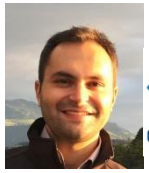


Next Steps:



- Finalize methodology
- Complete R2 review support & visualization
- Publish scientific paper
- Perform 4 TARs in ITCC-P4
- Make methodology & R2 access available to other groups

Nil Schubert



Guillaume Bergthold

Caitlin Lowery

Jan Koster

Outline

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  - ✓POC data wishlist
- POC data reviews (‘Target Actionability Reviews’)
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Subcategories per pediatric indication

Neuroblastoma
- Numerical chromosomal aberrations
- Segmental chromosomal aberrations:
TERT mutations
ATRX mutations
other mutations
- Myc amplified non segmental aberrations

Ewing sarcoma
- FET-ETS Ewing sarcoma with no other alteration
- FET-ETS Ewing sarcoma with STAG2 and/or TP53 and/or CDKN2A alterations
- Non FET-ETS Ewing like sarcoma (BCOR, CIC, NFACT2)

Rhabdomyosarcoma
- Alveolar RMS, PAX3-FKHR positive
- Alveolar RMS, PAX7-FKHR positive
- Embryonal RMS, RAS mut
- Embryonal RMS, RAS wt

Atypical Teratoid Rhabdoid Tumor (ATRT)
- ATRT-TYR
- ATRT-SHH
- ATRT-MYC
- Extra-cranial RT

HGG
- K27M mt
H3.3
H3.1
- G34 mt
- MYCN
- IGBM-RTK
ped RTK1
ped RTK2
- NOS: tumors that may not fall cleanly into one of the other groups

Medulloblastoma
- WNT MB
- SHH MB, TP53 wt
- SHH MB, TP53 mut
- Group 3 MB
- Group 4 MB

Ependymoma
- ST-EPN-RELA
- ST-EPN-YAP1
- PF-EPN-A
- PF-EPN-B

Osteosarcoma
No Subcategories

Non-RMS:
- MPNST
- Synovial sarcoma

NB pilot for minimally required POC datasets (within ITCCP4)

- 4 NB clinic-biological disease sub-categories
- For each clinic-biological category:
  - ✓ Scoring per POC data module
  - ✓ Define requirement for each data type within POC category

  (see Appendix 2 for detailed scores)

- Check scores with additional disease experts
- Derive ‘generalizable’ decision tree

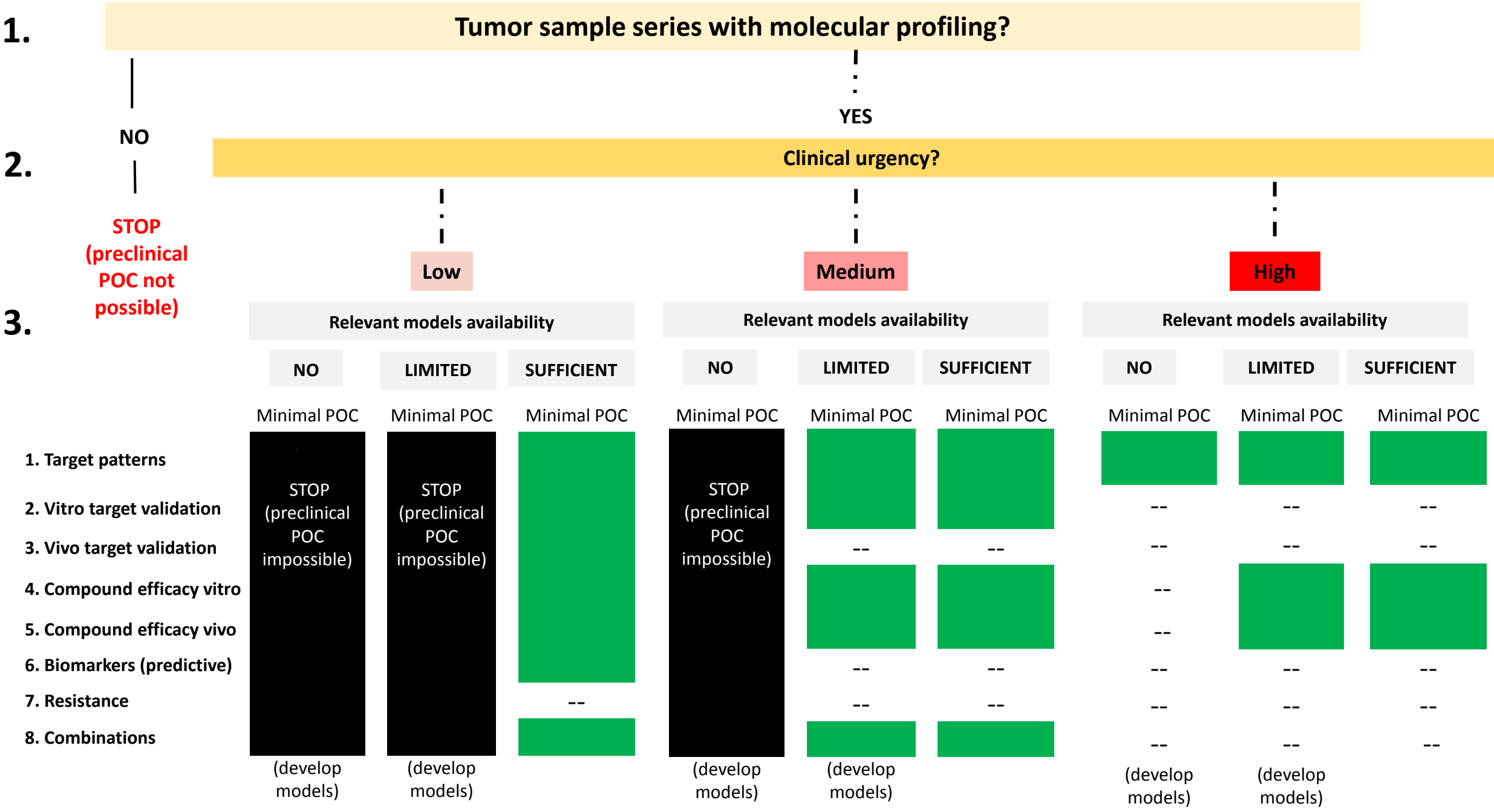
  (see next slide)

Minimal PoC package flow chart

Step 1: Availability of profiled tumor sample series

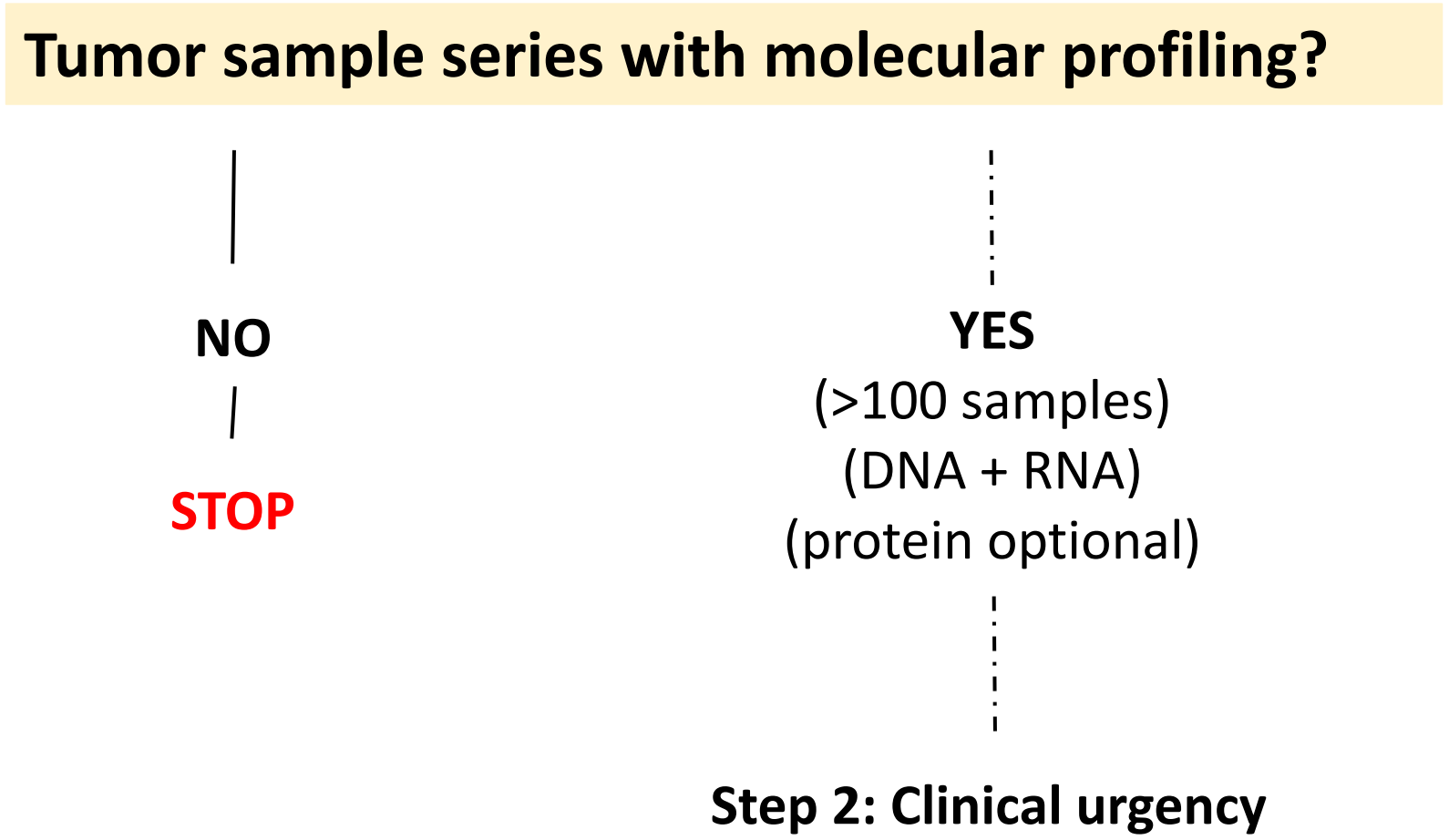
Step 2: Clinical urgency

Step 3: Availability of pediatric relevant models

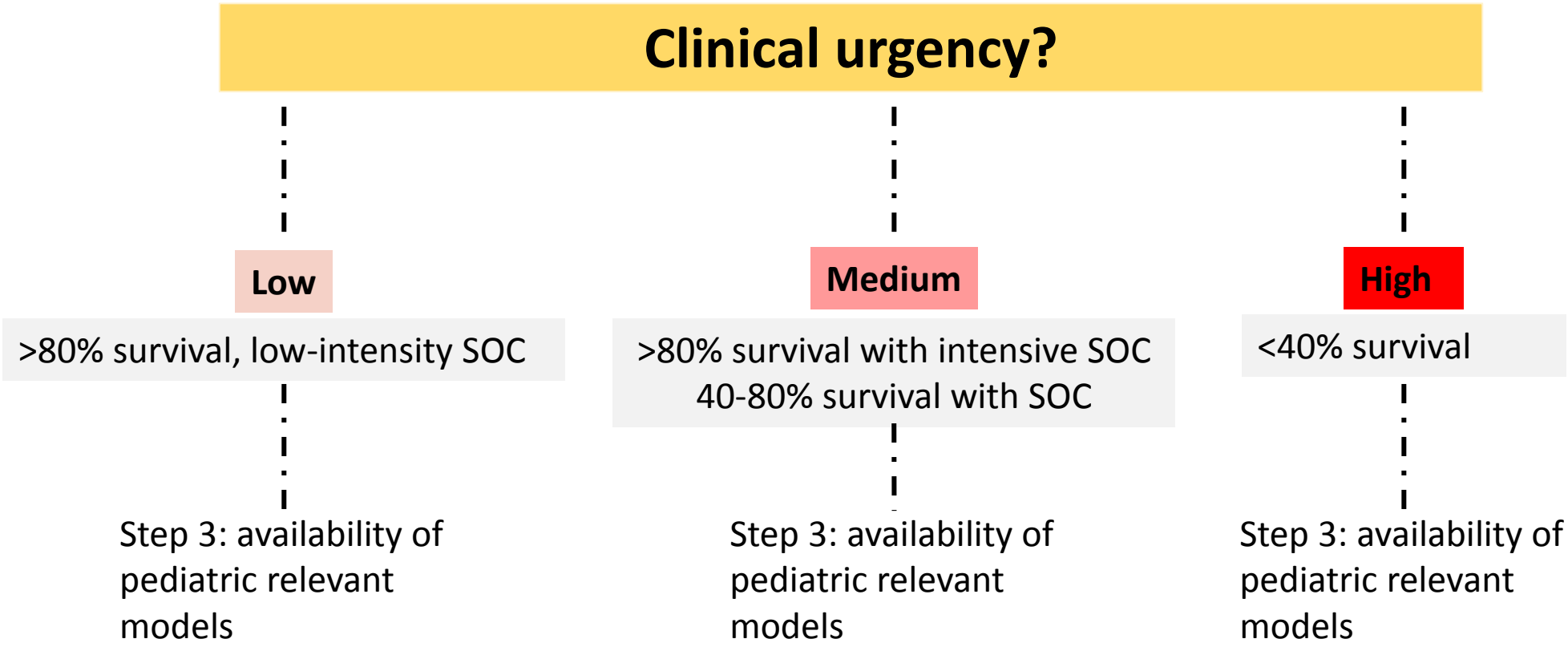

1.
Tumor sample series with molecular profiling?
NO
YES
2.
Clinical urgency?
STOP (preclinical POC not possible)
Low
Medium
High
3.
Relevant models availability
NO
LIMITED
SUFFICIENT
Minimal POC
1. Target patterns
2. Vitro target validation
3. Vivo target validation
4. Compound efficacy vitro
5. Compound efficacy vivo
6. Biomarkers (predictive)
7. Resistance
8. Combinations
STOP (preclinical POC impossible)
(develop models)

Minimal PoC package flow chart

Step 1: availability of profiled tumor sample series



Step 2: Clinical urgency



Step 3: availability of pediatric relevant models

LOW CLINICAL URGENCY

>80% survival, low-intensity SOC, good functional outcome

	Cell lines	Xenograft / PDX	Genetic models
NO	0	0	0
LIMITED	1-5	1-5	0-1
SUFFICIENT	>5	>5	>1

1. Target patterns
2. Vitro target validation
3. Vivo target validation
4. Compound efficacy vitro
5. Compound efficacy vivo
6. Biomarkers (predictive)
7. Resistance
8. Combinations

Relevant models availability

NO

Minimal POC

STOP (preclinical POC impossible)

(develop models)

LIMITED

Minimal POC

STOP (preclinical POC impossible)

(develop models)

SUFFICIENT

Minimal POC

--

Step 3: availability of pediatric relevant models

	Cell lines	Xenograft / PDX	Genetic models
NO	0	0	0
LIMITED	1-5	1-5	0-1
SUFFICIENT	>5	>5	>1

MEDIUM CLINICAL URGENCY

>80% survival with intensive SOC , 40-80% survival with SOC, major functional impairments

Relevant models availability

NO

Minimal POC

1. Target patterns
2. Vitro target validation
3. Vivo target validation
4. Compound efficacy vitro
5. Compound efficacy vivo
6. Biomarkers (predictive)
7. Resistance
8. Combinations

STOP (preclinical POC impossible)

(develop models)

LIMITED

Minimal POC

--

--

--

(develop models)

SUFFICIENT

Minimal POC

--

--

--

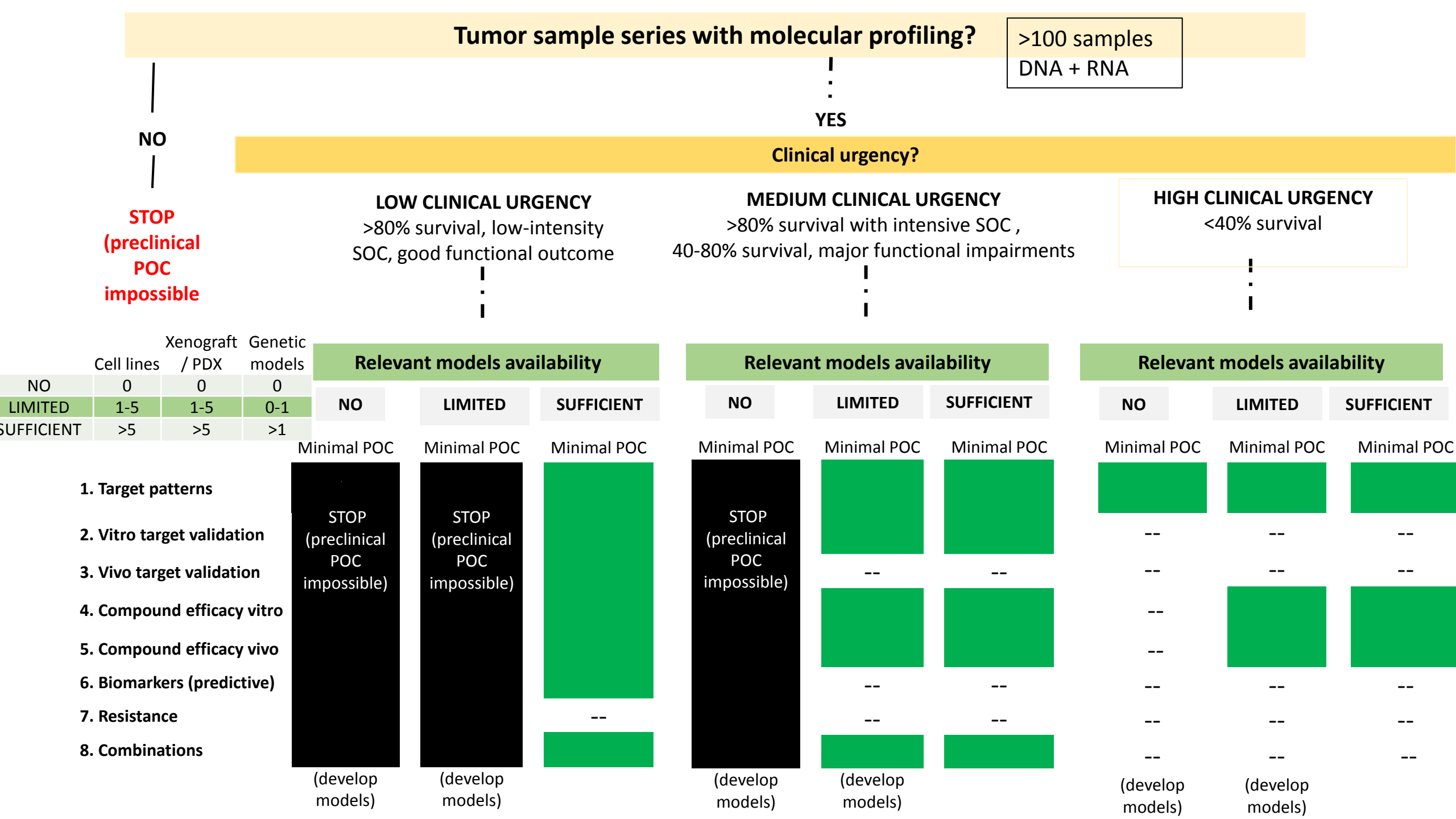
Step 3: availability of pediatric relevant models

	Cell lines	Xenograft / PDX	Genetic models
NO	0	0	0
LIMITED	1-5	1-5	0-1
SUFFICIENT	>5	>5	>1

HIGH CLINICAL URGENCY
<40% survival

Relevant models availability

	NO	LIMITED	SUFFICIENT
Minimal POC	1. Target patterns	1. Target patterns	1. Target patterns
1. Target patterns	*	*	**
2. Vitro target validation	--	--	--
3. Vivo target validation	--	--	--
4. Compound efficacy vitro	--		
5. Compound efficacy vivo	--		
6. Biomarkers (predictive)	--	--	--
7. Resistance	--	--	--
8. Combinations	--	--	--
	(develop models)	(develop models)	


Tumor sample series with molecular profiling?
>100 samples
DNA + RNA
NO
YES
STOP (preclinical POC impossible
Clinical urgency?
LOW CLINICAL URGENCY
>80% survival, low-intensity SOC, good functional outcome
MEDIUM CLINICAL URGENCY
>80% survival with intensive SOC , 40-80% survival, major functional impairments
HIGH CLINICAL URGENCY
<40% survival
Cell lines
Xenograft / PDX
Genetic models
NO 0 0 0
LIMITED 1-5 1-5 0-1
SUFFICIENT >5 >5 >1
Relevant models availability
NO
LIMITED
SUFFICIENT
Minimal POC
1. Target patterns
2. Vitro target validation
3. Vivo target validation
4. Compound efficacy vitro
5. Compound efficacy vivo
6. Biomarkers (predictive)
7. Resistance
8. Combinations
STOP (preclinical POC impossible)
(develop models)

Discussion:

POC data content:

- Subcategories
- Use of clinically relevant dosing schedules (NMEs and SOC)
- Combination testing
- BM testing

Minimally required POC datasets:

- Steps
- Cut-offs

Minimal PoC package flow chart

Step 1: Availability of profiled tumor sample series

>100 samples
DNA + RNA

Step 2: Clinical urgency

LOW CLINICAL URGENCY
>80% survival, low-intensity SOC, good functional outcome

MEDIUM CLINICAL URGENCY
>80% survival with intensive SOC ,
OR
40-80% survival with SOC, major functional impairments

HIGH CLINICAL URGENCY
<40% survival

Step 3: Availability of pediatric relevant models

	Cell lines	Xenograft / PDX	Genetic models
NO	0	0	0
LIMITED	1-5	1-5	0-1
SUFFICIENT	>5	>5	>1

Appendix 1

POC data categories and data types

SIOP Taskforce 2014

1. Target status and patterns in clinical series

Sample series	at diagnosis	>100 samples/tumor type with at least 10 samples/ clinic-biological subset
	clinical annotation	correlation with clinically-used risk stratification
	biological annotation	Use published validated biological characterization technologies (DNA methylation, EG or others)
Tissue types		single biopsy at diagnosis
Target aberrations	DNA	Translocations
		Mutation characteristic signatures (TMB status, BRCA, EG)
		CNV
	mRNA	single gene or pathway signature (target dependent) compared to ped cancers + adult cancers + normal tissues
		single gene or pathway signature (target dependent) compared to ped cancers + adult cancers

2. Molecular validation of tumor dependence on the target in vitro

Model systems	Cell lines (established)
	Patient derived (short term)
Downregulation	RNA interference (siRNA, shRNA, inducible shRNA)
PD readouts ('on target' biological effects)	Pathway modulation (incl target protein levels) (eg pERK)
	Biological effect (e.g. apoptosis, senescence, differentiation)
Phenotype readout	Tumor cell viability (eg. MTT)

3. Molecular validation of target dependence in vivo

Model systems	Mouse xenograft sc
	Mouse xenograft orthotopic
	Mouse PDX sc
	Mouse PDX orthotopic
	Transgenic mice
	Metastatic model
Downregulation	RNA interference (siRNA, shRNA, inducible shRNA) vivo knockdown
Upregulation	Transgenic
	Tumor response (CR, PR, SD) (serial time measurements)
	Survival (PFS, OS)
PD readouts ('on target' biological effects)	Target binding
	Target inhibition
	Pathway modulation (eg. pERK)
	Biological effect (e.g. apoptosis, senescence, differentiation, cell cycle arrest)

4. Compound efficacy in vitro models

Model systems	Cell lines (established)
	Patient derived (short term)
PD readouts	Pathway modulation (eg. pERK)
	Biological effect (e.g. cell cycle arrest, apoptosis, senescence, differentiation)
Phenotypic efficacy readout	Tumor cell viability (eg. MTT)

5. Compound efficacy in vivo models

Model systems	Extracranial tumors : PDX sc Intracranial tumors: Orthotropic PD
Efficacy readout	Tumor response (CR, PR, SD) (serial time measurements)
PK (systematic plus intratumoral)	Use of clinically relevant dosing schedule

6. Target efficacy relationship (biomarker)

Predictive (patient selection)	Is a test for a predictive BM available for the target (pathway)
	Does the aberration reflected by the pred.BM occur in pediatric tumors of interest
	Assay for pred.BM is technically validated, as diagnostic test for clinical and/or research use
	BM correlates with efficacy of targeted compound in pre-clinical models of pediatric tumor of interest

7. Resistance

Models	vitro resistance models available, e.g. by longterm treatment
	vivo resistance models available, e.g. by longterm treatment
Mechanisms	resistance mechanism in vitro models available
	resistance mechanism in vivo models available
Molecular Validation	validate candidate resistance pathways by molecular intervention
	validate candidate resistance pathways by drug intervention

8. Combinations

Vitro models	readout: cell death/ proliferation
	combination-Index methodology (synergistic, additive, antagonistic)
Vivo models (efficacy)	readout: tumor response
	readout: survival
Vivo models (toxicity, therapeutic index)	dosing schedules (clinically relevant)

Appendix 2

Neuroblastoma minimal POC package

ITCCP4 NB experts, 2018

1. Target status and patterns in clinical series

			Numerical chromosomal aberrations	Segmental chromosomal aberrations			Myc ampl non segmental aberrations
				ATRX	TERT	Other	
1A. Sample series	at diagnosis	>100 samples/tumor type with at least 10 samples/ clinico-biological subset	✔	✔	✔	✔	✔
	clinical annotation	correlation with clinically-used risk stratification	✔	✔	✔	✔	✔
		correlation with outcome					
1B. Tissue types		single biopsy	✔	✔	✔	✔	✔
		multiple biopsy from same tumor					
		multiple biopsies in time					
		metastases					
1C. Target aberrations	DNA	Translocations	✔	✔	✔	✔	✔
		Mut: insertion/delection	✔	✔	✔	✔	✔
		CNV focal >8	✔	✔	✔	✔	✔
		CNV regional <4	✔	✔	✔	✔	✔
		methylation patterns					
	mRNA	single gene or pathway signature (target dependent) compared to ped cancers + adult cancers + normal tissues	✔	✔	✔	✔	✔
		single gene or pathway signature (target dependent) compared to ped cancers + adult cancers	✔	✔	✔	✔	✔
	Protein	single target ped + adult/normal					
		pathway signature					
	miRNA	single target ped + adult/normal					

2. Molecular validation of tumor dependence on the target in vitro

		Numerical chromosomal aberrations	Segmental chromosomal aberrations			Mycn ampl non segmental aberrations
			ATRX	TERT	Other	
Model systems	Cell lines (established)	✔	✔	✔	✔	✔
	Patient derived (short term)	✔	✔	✔	✔	✔
	3D cultures					
Culture conditions	Hypoxia					
	Other environmental stress					
Downregulation	RNA interference (siRNA, shRNA, inducible shRNA)	✔	✔	✔	✔	✔
	somatic knockouts					
Upregulation	transfection					
	somatic knock in					
PD readouts ('on target' biological effects)	Pathway modulation (incl target protein levels) (eg pERK)	✔	✔	✔	✔	✔
	Biological effect (e.g. apoptosis, senescence, differentiation)	✔	✔	✔	✔	✔
Phenotype readout	Tumor cell viability (eg. MTT)	✔	✔	✔	✔	✔
	Tumor cell death					
	Tumor cell proliferation					
	Clonogenicity					

3. Molecular validation of target dependence in vivo

		Numerical chromosomal aberrations	Segmental chromosomal aberrations			My ampl non segmental aberrations
			ATRX	TERT	Other	
Model systems	Mouse xenograft sc	no data needed in minimally required POC dataset to support clincal trial development				
	Mouse xenograft orthotopic					
	Mouse PDX sc					
	Mouse PDX orthotopic					
	Transgenic mice					
	Metastatic model					
	Endogenous animal models reflecting human disease					
	Other animal models (eg. Zebrafish)					
Downregulation	RNA interference (siRNA, shRNA, inducible shRNA) vivo knockdown					
	somatic knockouts					
Upregulation	Transgenic					
	Knock-in					
Efficacy readout	tumor growth inhibition					
	Tumor response (CR, PR, SD) (serial time measurements)					
	Survival (PFS, OS)					
PD readouts ('on target' biological effects)	Target binding					
	Target inhibition					
	Pathway modulation (eg. pERK)					
	Biological effect (e.g. apoptosis, senescence, differentiation, cell cycle arrest)					

4. Compound efficacy in vitro models

		Numerical chromosomal aberrations	Segmental chromosomal aberrations			Mycn ampl non segmental aberrations
			ATRX	TERT	Other	
Model systems	Cell lines (established)	✔	✔	✔	✔	✔
	Patient derived (short term)	✔	✔	✔	✔	✔
	Ex-vivo co cultures					
	Slices					
	3D cultures					
Culture conditions	Hypoxia					
	Other environmental stress					
PD readouts	Target binding					
	Target inhibition					
	Pathway modulation (eg. pERK)	✔	✔	✔	✔	✔
	Biological effect (e.g. cell cycle arrest, apoptosis, senescence, differentiation)	✔	✔	✔	✔	✔
Phenotypic efficacy readout	Tumor cell viability (eg. MTT)	✔	✔	✔	✔	✔
	Tumor cell death					
	Tumor cell proliferation					
	Clonogenicity					

5. Compound efficacy in vivo models

		Numerical chromosomal aberrations	Segmental chromosomal aberrations			Mycn ampl non segmental aberrations
			ATRX	TERT	Other	
Model systems:	Mouse xenograft sc					
	Mouse xenograft orthotopic					
	Mouse PDX sc	✔	✔	✔	✔	✔
	Mouse PDX orthotopic					
	Transgenic mice					
	Metastatic model					
	Endogenous animal models reflecting human disease					
	Other animal models (e.g. Zebrafish)					
	Control models					
Efficacy readout	tumor growth inhibition					
	Tumor response (CR, PR, SD) (serial time measurements)	✔	✔	✔	✔	✔
	Survival (PFS, OS)					
PD readouts ('on target' biological effects)	Target binding					
	Target inhibition					
	Pathway modulation (eg. pERK)					
	Biological effect (e.g. apoptosis, senescence, differentiation, cell cycle arrest)					
PK (systematic plus intratumoral)	Use of clinically relevant dosing schedule	✔	✔	✔	✔	✔
	Correlation serum - intratumor					
	Serum drug concentrations required for tumor response					
	Serum Drug concentrations required for target inhibition+ modulation					
	Intratumor drug concentrations required for tumor response					
	Intratumor drug concentrations required for target inhibition+ modulation					
PK-PD relation	Minimal level and duration of target inhibition required to achieve anti-tumor efficacy					

6. Target efficacy relationship (biomarker)

		Numerical chromosomal aberrations	Segmental chromosomal aberrations			My ampifiedl non segmental aberrations
			ATRX	TERT	Other	
6A. Predictive (patient selection)	Is a test for a predictive BM available for the target (pathway)	✔	✔	✔	✔	✔
	Does the aberration reflected by the pred.BM occur in pediatric tumors of interest	✔	✔	✔	✔	✔
	Assay for pred.BM is technically validated, as diagnostic test for clinical and/or research use	✔	✔	✔	✔	✔
	BM correlates with efficacy of targeted compound in pre-clinical models of pediatric tumor of interest	✔	✔	✔	✔	✔
	BM correlates with efficacy of targeted compound in clinical trials in pediatric tumor of interest					
	Pred.BM is clinically validated in pediatric tumor of interest					
	Pred.BM is clinically validated in adult clinical trials					
6B. PD (biological efficacy)	Is a test available for biological efficacy of target inhibition by targeted compound					
6C. Surrogate clinical efficacy	use clinically established surrogate/ intermediate endpoints					

7. Resistance

		Numerical chromosomal aberrations	Segmental chromosomal aberrations			My ampl non segmental aberrations
			ATRX	TERT	Other	
Models	vitro resistance models available, e.g. by longterm treatment					
	vivo resistance models available, e.g. by longterm treatment					
Mechanisms	resistance mechanism in vitro models available					
	resistance mechanism in vivo models available					
Molecular Validation	validate candidate resistance pathways by molecular intervention					
	validate candidate resistance pathways by drug intervention					

8. Combinations

		Numerical chromosomal aberrations	Segmental chromosomal aberrations			My ampl non segmental aberrations
			ATRX	TERT	Other	
8A. vitro models	readout: cell death/ proliferation	✔	✔	✔	✔	✔
	combination-Index methodology (synergistic, additive, antagonistic)	✔	✔	✔	✔	✔
	dosing schedules (clinically relevant)	✔	✔	✔	✔	✔
8B. vivo models (efficacy)	readout: tumor response	✔	✔	✔	✔	✔
	readout: survival	✔	✔	✔	✔	✔
8C. Vivo models (toxicity, therapeutic index)	dosing schedules (clinically relevant)	✔	✔	✔	✔	✔
	readout: therapeutic index (antitumor effect vs normal tissue toxicity)	not in minimal dataset				
	increased antitumor / decreased toxicity					
	dosing schedules (clinically relevant)					

Feedback from disease experts:

- Feedback received for the following indications: MD, HGG and Ependimoma
- Incorporation of blood brain penetrance assessment to the in vivo PoC package

Medulloblastoma

	WNT MB	SHH MB, TP53 wt	SHH MB, TP53 mt	Group 3	Group 4
Step 1: availability of profiled tumor sample series	Yes	Yes	Yes	Yes	Yes
Step 2: Clinical urgency	Medium	High	Medium	High	Medium
Step 3: availability of pediatric relevant models	Limited	Limited	Limited	Sufficient	Limited

Ependymoma

	ST-EPN-RELA	ST-EPN-YAP1	PF-EPN-A	PF-EPN-B
Step 1: availability of profiled tumor sample series	Yes	Yes	Yes	Yes
Step 2: Clinical urgency	High	Medium	High	Medium
Step 3: availability of pediatric relevant models	Limited	Limited	Limited	No

HGG	K27 mut		G34 mut	MYCN	IGBM-RTK		Non other Subcat.
	H3.3	H3.1			RTK1	RTK2	
Step 1: availability of profiled tumor sample series	Yes	Yes	Yes	Yes	Yes	Yes	(Yes)
Step 2: Clinical urgency	High	Hig	High	High	High	High	High
Step 3: availability of pediatric relevant models	Sufficient	Limited	Limited	Limited	Limited	Lim/NO	Limited

ATRT

	ATRT-TYR	ATRT-SHH	ATRT-MYC	Extra-cranial RT
Step 1: availability of profiled tumor sample series	Yes	Yes	Yes	Yes
Step 2: Clinical urgency	High	High	High	High
Step 3: availability of pediatric relevant models	Limited	Limited	Limited	Limited