Convening Experts in Oncology to Address Children’s Health

Quarterly Collaboration Meetings in Pediatric Oncology

March 15, 2023 | Virtual Meeting

Reviewed Targets:

ATR
HDAC
PIK3 alpha

This meeting summary was prepared by Rose Li and Associates, Inc., under contract to The Foundation for the National Institutes of Health (FNIH). The views expressed in this document reflect both individual and collective opinions of the meeting participants and not necessarily those of FNIH. Review of earlier versions of this meeting summary by the following individuals are gratefully acknowledged: Kelly E. Beazley and Gina Castelvecchi.

Target assessment was prepared by Citeline (formerly Informa Pharma Intelligence), under the contract to the Foundation for the National Institutes of Health (FNIH). The following individuals are gratefully acknowledged: Markella Kordoyanni, Stephanie Yip, James Drew, Tobias Brady, Amanda Micklus, Astrid Kurniawan, JP Park, Kripa Krishnan.
**Acronym Definitions**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AACR</td>
<td>American Association for Cancer Research</td>
</tr>
<tr>
<td>ADC</td>
<td>antibody drug conjugates</td>
</tr>
<tr>
<td>ADCC</td>
<td>antibody-dependent cellular cytotoxicity</td>
</tr>
<tr>
<td>AE</td>
<td>adverse event</td>
</tr>
<tr>
<td>AKT</td>
<td>AKT serine/threonine kinase</td>
</tr>
<tr>
<td>ALL</td>
<td>acute lymphocytic leukemia</td>
</tr>
<tr>
<td>AML</td>
<td>acute myeloid leukemia</td>
</tr>
<tr>
<td>APC</td>
<td>adenomatous polyposis coli</td>
</tr>
<tr>
<td>ARMS</td>
<td>alveolar rhabdomyosarcoma</td>
</tr>
<tr>
<td>ATM</td>
<td>ataxia telangiectasia mutated</td>
</tr>
<tr>
<td>ATR</td>
<td>ataxia telangiectasia and Rad3-related protein</td>
</tr>
<tr>
<td>ATRIP</td>
<td>ataxia telangiectasia and Rad3-related-interacting protein</td>
</tr>
<tr>
<td>ATRT</td>
<td>atypical teratoid rhabdoid tumor</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>B-ALL</td>
<td>B-cell acute lymphocytic leukemia</td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>BCL-2</td>
<td>B-cell lymphoma 2</td>
</tr>
<tr>
<td>BCL-XL</td>
<td>B-cell lymphoma-extra large</td>
</tr>
<tr>
<td>BRCA2</td>
<td>breast cancer 2</td>
</tr>
<tr>
<td>BRD4</td>
<td>bromodomain-containing protein 4</td>
</tr>
<tr>
<td>CCSK</td>
<td>clear cell sarcoma of the kidney</td>
</tr>
<tr>
<td>CDK</td>
<td>cyclin-dependent kinase</td>
</tr>
<tr>
<td>CDX</td>
<td>cell line-derived xenograft</td>
</tr>
<tr>
<td>CEBPA</td>
<td>CCAAT/enhancer-binding protein alpha</td>
</tr>
<tr>
<td>CBFβ</td>
<td>core-binding factor subunit beta</td>
</tr>
<tr>
<td>CLL</td>
<td>chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CNV</td>
<td>copy number variation</td>
</tr>
<tr>
<td>COACH</td>
<td>Convoking Experts in Oncology to Address Children’s Health</td>
</tr>
<tr>
<td>CR</td>
<td>complete remission</td>
</tr>
<tr>
<td>DCR</td>
<td>disease control rate</td>
</tr>
<tr>
<td>DepMap</td>
<td>Dependency Map</td>
</tr>
<tr>
<td>DIPG</td>
<td>diffuse intrinsic pontine glioma</td>
</tr>
<tr>
<td>DLBCL</td>
<td>diffuse large B cell lymphoma</td>
</tr>
<tr>
<td>DLT</td>
<td>dose-limiting toxicity</td>
</tr>
<tr>
<td>DMG</td>
<td>diffuse midline glioma</td>
</tr>
<tr>
<td>DSRCT</td>
<td>desmoplastic small round cell tumor</td>
</tr>
<tr>
<td>EAE</td>
<td>experimental autoimmune encephalomyelitis</td>
</tr>
<tr>
<td>EBV</td>
<td>Epstein Barr Virus</td>
</tr>
<tr>
<td>EFS</td>
<td>event-free survival</td>
</tr>
<tr>
<td>EGFR</td>
<td>epidermal growth factor</td>
</tr>
<tr>
<td>EMA</td>
<td>European Medicines Agency</td>
</tr>
<tr>
<td>EMT</td>
<td>epithelial-mesenchymal transition</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>ERMS</td>
<td>embryonal rhabdomyosarcoma</td>
</tr>
</tbody>
</table>
Convening Experts in Oncology to Address Children’s Health
March 15, 2023

EwS  Ewing sarcoma
FANCl  Fanconi anemia complementation group I
FDA  Food and Drug Administration
FOXO1  forkhead box protein O1
GBM  glioblastoma multiforme
GVHD  graft-versus-host disease
HAT  histone acetyltransferase
HDAC  histone deacetylase
HGG  high-grade glioma
HIV  human immunodeficiency virus
HL  Hodgkin’s lymphoma
HSC  hematopoietic stem cell
ICI  immune checkpoint inhibitor
IDH  isocitrate dehydrogenase
IFNg  interferon gamma
IGF  insulin-like growth factor
IHC  immunohistochemical
ITCC-P4  Paediatric Preclinical Proof of Concept Platform
JAK  Janus kinase
kg  Kilogram
LOF  loss-of-function
M  Molar
mAb  monoclonal antibody
MAPK  mitogen-activated protein kinase
MCL-1  myeloid cell leukemia 1
mCR  maintained complete response
mDOR  median duration of response
MEK  mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase
MIBG  meta-iodobenzylguanidine
MLL  mixed-lineage leukemia
MNST  malignant nerve sheath tumor
MPNST  malignant peripheral nerve sheath tumor
MRT  malignant rhabdoid tumor
MTD  maximum tolerated dose
NAD+  nicotinamide adenine dinucleotide
NAMPT  nicotinamide phosphoribosyltransferase
NCE  new chemical entity
NCI  National Cancer Institute
nHL  non-Hodgkin’s lymphoma
NIDDK  National Institute of Diabetes and Digestive and Kidney Diseases
NIH  National Institutes of Health
NSCLC  non-small cell lung cancer
ODAC  Oncologic Drugs Advisory Committee
OR  objective response
ORR  overall response rate
OS  overall survival
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP1</td>
<td>poly [ADP-ribose] polymerase 1</td>
</tr>
<tr>
<td>PAX3</td>
<td>paired box gene 3</td>
</tr>
<tr>
<td>PBD</td>
<td>Pyrrolobenzodiazepine</td>
</tr>
<tr>
<td>PD</td>
<td>progressive disease</td>
</tr>
<tr>
<td>PDGF</td>
<td>platelet-derived growth factor</td>
</tr>
<tr>
<td>PDX</td>
<td>patient-derived xenograft</td>
</tr>
<tr>
<td>PFS</td>
<td>progression-free survival</td>
</tr>
<tr>
<td>PGBD5</td>
<td>PiggyBac transposable element-derived 5</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphatidylinositol 3-kinase</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha</td>
</tr>
<tr>
<td>PIP2</td>
<td>phosphatidylinositol 4,5-biphosphate</td>
</tr>
<tr>
<td>PIP3</td>
<td>phosphatidylinositol (3,4,5)-triphosphate</td>
</tr>
<tr>
<td>PIVOT</td>
<td>Pediatric Preclinical In Vivo Testing</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
</tr>
<tr>
<td>PPP</td>
<td>public-private partnership</td>
</tr>
<tr>
<td>PPTC</td>
<td>Pediatric Preclinical Testing Consortium</td>
</tr>
<tr>
<td>PPTP</td>
<td>Pediatric Preclinical Testing Program</td>
</tr>
<tr>
<td>PR</td>
<td>partial response</td>
</tr>
<tr>
<td>PRC2</td>
<td>polycomb repressive complex 2</td>
</tr>
<tr>
<td>PRISM</td>
<td>Profiling Relative Inhibition Simultaneously in Mixtures</td>
</tr>
<tr>
<td>PROS</td>
<td>PIK3CA-related overgrowth spectrum</td>
</tr>
<tr>
<td>PROTAC</td>
<td>proteolysis targeting chimera</td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatase and tensin homolog</td>
</tr>
<tr>
<td>QTc</td>
<td>corrected QT interval</td>
</tr>
<tr>
<td>RMS</td>
<td>rhabdomyosarcoma</td>
</tr>
<tr>
<td>RP2D</td>
<td>recommended Phase II dose</td>
</tr>
<tr>
<td>RPA</td>
<td>replication protein A</td>
</tr>
<tr>
<td>RTK</td>
<td>receptor tyrosine kinase</td>
</tr>
<tr>
<td>SD</td>
<td>stable disease</td>
</tr>
<tr>
<td>SMARCAL1</td>
<td>switch/sucrose non-fermentable related, matrix associated, actin dependent regulator of chromatin, subfamily a like 1</td>
</tr>
<tr>
<td>SME</td>
<td>subject matter expert</td>
</tr>
<tr>
<td>SMMHC</td>
<td>smooth muscle myosin heavy chain</td>
</tr>
<tr>
<td>SP1</td>
<td>specificity protein 1</td>
</tr>
<tr>
<td>ssDNA</td>
<td>single-stranded DNA</td>
</tr>
<tr>
<td>SSM</td>
<td>simple somatic mutation</td>
</tr>
<tr>
<td>SV</td>
<td>structural variations</td>
</tr>
<tr>
<td>SWI/SNF</td>
<td>switch/sucrose non-fermentable</td>
</tr>
<tr>
<td>T-ALL</td>
<td>T-cell ALL</td>
</tr>
<tr>
<td>TEAE</td>
<td>treatment emergent adverse event</td>
</tr>
<tr>
<td>TMB</td>
<td>tumor mutational burden</td>
</tr>
<tr>
<td>TME</td>
<td>tumor microenvironment</td>
</tr>
<tr>
<td>TNBC</td>
<td>triple-negative breast cancer</td>
</tr>
<tr>
<td>WRN</td>
<td>Werner syndrome ATP-dependent helicase</td>
</tr>
</tbody>
</table>
## Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acronym Definitions</td>
<td>ii</td>
</tr>
<tr>
<td>Executive Summary</td>
<td>6</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>7</td>
</tr>
<tr>
<td>Key Considerations</td>
<td>7</td>
</tr>
<tr>
<td>Next Steps</td>
<td>7</td>
</tr>
<tr>
<td>ATR</td>
<td>8</td>
</tr>
<tr>
<td>Key Considerations</td>
<td>8</td>
</tr>
<tr>
<td>Next Steps</td>
<td>8</td>
</tr>
<tr>
<td>HDAC 1-3</td>
<td>9</td>
</tr>
<tr>
<td>Key Considerations</td>
<td>9</td>
</tr>
<tr>
<td>Next Steps</td>
<td>10</td>
</tr>
<tr>
<td>Meeting Summary</td>
<td>11</td>
</tr>
<tr>
<td>Review of Meeting Objectives</td>
<td>11</td>
</tr>
<tr>
<td>Pediatric Cancer Drug Target Data</td>
<td>11</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>12</td>
</tr>
<tr>
<td>Overview</td>
<td>12</td>
</tr>
<tr>
<td>Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival</td>
<td>12</td>
</tr>
<tr>
<td>Dependency and Drug Sensitivity</td>
<td>13</td>
</tr>
<tr>
<td>Clinical Trial Development</td>
<td>15</td>
</tr>
<tr>
<td>Research and Development Landscape</td>
<td>15</td>
</tr>
<tr>
<td>Additional Considerations for Preclinical and Clinical Development</td>
<td>15</td>
</tr>
<tr>
<td>Toxicity Concerns</td>
<td>16</td>
</tr>
<tr>
<td>Central Nervous System Tumor Complexity and Crossing the Blood-Brain Barrier</td>
<td>16</td>
</tr>
<tr>
<td>Rational Treatment Combinations</td>
<td>16</td>
</tr>
<tr>
<td>Patient Selection</td>
<td>17</td>
</tr>
<tr>
<td>Next Steps</td>
<td>17</td>
</tr>
<tr>
<td>ATR</td>
<td>17</td>
</tr>
<tr>
<td>Overview</td>
<td>17</td>
</tr>
<tr>
<td>Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival</td>
<td>17</td>
</tr>
<tr>
<td>Dependency and Drug Sensitivity</td>
<td>18</td>
</tr>
<tr>
<td>Clinical Trial Development</td>
<td>19</td>
</tr>
<tr>
<td>Research and Development Landscape</td>
<td>19</td>
</tr>
<tr>
<td>Additional Considerations for Preclinical and Clinical Development</td>
<td>20</td>
</tr>
<tr>
<td>Biomarker Identification</td>
<td>20</td>
</tr>
<tr>
<td>Combination Therapies</td>
<td>20</td>
</tr>
<tr>
<td>Treatment of Relapsed Versus Untreated Tumors</td>
<td>21</td>
</tr>
</tbody>
</table>
Executive Summary

Despite advances in therapeutic development for adult cancers, developing treatment regimens for pediatric cancers poses unique challenges; effective adult treatments are not always readily translatable to the pediatric population due to distinct differences between adults and children, even those with the same cancer diagnosis. In addition, pediatric cancer patient populations are quite small, which complicates study design and sufficient powering for pediatric clinical trials. Convening Experts in Oncology to Address Children’s Health (COACH) assembles subject matter experts (SMEs) from diverse fields to review research landscapes for therapeutic targets of potential interest for pediatric oncology indications and offer recommendations regarding preclinical research needed to further develop existing therapeutics for use in pediatric populations. On March 15, 2023, COACH convened the Fourth Quarterly Collaboration Meeting—with SMEs from the National Cancer Institute (NCI), Food and Drug Administration (FDA), European Medicines Agency (EMA), advocacy groups, the pharmaceutical industry, Paediatric Preclinical Proof of Concept Platform (ITCC-P4), and the Pediatric Preclinical In Vivo Testing (PIVOT) consortium--to discuss and provide recommendations regarding preclinical research required to develop the following drug targets for early phase pediatric clinical trials: phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), ataxia telangiectasia and Rad3-related protein (ATR), and histone deacetylase (HDAC) 1-3.
PIK3CA

PIK3CA is an important component of the phosphatidylinositol 3-kinase (PI3K)/AKT serine/threonine kinase (AKT) signaling pathway, which regulates both cell growth and survival. While PIK3CA mutations are uncommon in pediatric cancers, they are observed in appreciable numbers for a subset of central nervous system (CNS) tumors, such as diffuse intrinsic pontine gliomas (DIPGs) and non-brain stem high-grade gliomas (HGGs). Notably, while 17.5 percent of DIPG tumors have PIK3CA mutations, the variant allele frequencies are generally low, suggesting these mutations may be subclonal and not causative. PIK3CA is generally expressed at lower levels in pediatric cancer cell lines compared to adult cancer cell lines, but genetic loss of PIK3CA negatively impacts cell viability across all pediatric tumor types. However, treatment of cell lines with alpelisib (a PI3K inhibitor) affected only malignant rhabdoid tumor (MRT) cell lines in the PRISM drug screen, and PI3K inhibitors have limited single-agent activity in leukemia and solid tumor mouse models, with only glioma mouse models responding (modestly) to single-agent PIK3CA inhibition.

In a clinical trial of the PIK3CA inhibitor copanlisib for pediatric patients with relapsed or refractory solid tumors, responses were not observed in the phase 1 component. Results for the phase 2 component are not yet available. However, targeting related signaling pathways (e.g., Janus kinase [JAK]) in combination with PIK3CA inhibition may result in beneficial synergistic effects. Notably, although PI3KCA is required for embryogenesis, blood glucose homeostasis, and reproductive organ function, the only treatment emergent adverse events (TEAEs) observed in a Phase I/II pediatric trial of copanlisib were hyperglycemia, nausea, and decreased white blood cell count (Bayer, 2023). Currently, two inhibitors active against PIK3CA are FDA-approved (copanlisib and alpelisib), and 33 PI3K-targeting agents are in development.

Key Considerations

- PIK3CA inhibitors have variable penetrance of the blood-brain barrier (BBB), which affects treatment efficacy of CNS tumors as well as side effect profiles.
- While PIK3CA inhibitors have been effective in preclinical CNS tumor models, CNS-penetrant PIK3CA inhibitors may have neuropsychiatric toxicities in human patients.
- Data from PIK3CA-related overgrowth spectrum (PROS) patients treated with alpelisib may provide insights relevant to pediatric oncology indications.
- Synergistic treatment combinations may also have synergistic toxicities.
- Identification of biomarkers that predict PIK3CA inhibitor sensitivity is essential prior to any additional PIK3CA inhibitor pediatric clinical trials.

Next Steps

- PIK3CA inhibition is a low priority target for further preclinical development at this time as reviewed by COACH.
- Results from pending adult clinical trials may provide insight into potentially relevant pediatric populations as well as rational treatment combinations, such as those targeting tumor escape mechanisms
- Mechanistic studies to better understand CNS toxicities are needed.
• Preclinical development of predictive biomarkers for PIK3CA sensitivity.

**ATR**

ATR is a component of the DNA damage response pathway, and dysregulation of this response can drive tumorigenesis. While ATR mutations are very rare in pediatric patients, mutations in ataxia-telangiectasia mutated (ATM) and other DNA repair pathway mutations may sensitize tumors to ATR inhibition. Notably, all pediatric cancer cell lines are sensitive to genetic loss of ATR, but this responsiveness is more moderate for pharmacological inhibition. ATR inhibitors have been effective in leukemia, sarcoma, and glioma mouse models, but their efficacies in other solid tumor models is not well-established. Low expression of ATR is required for embryogenesis and tissue homeostasis, so ATR inhibition treatment strategies will require appropriate dosing to minimize toxic effects.

Currently, there are no ongoing or planned pediatric-specific ATR inhibitor Phase III clinical trials. Clinical trials in the United States that include pediatric patients are assessing ceralasertib plus olaparib in osteosarcoma (Phase II) and elimusertib in Ewing sarcoma (EwS) or alveolar rhabdomyosarcoma (Phase I/II), although no results are available (Dana-Farber Cancer Institute, 2022; National Cancer Institute (NCI), 2023a). A European proof-of-concept Phase I/II trial of 18 pediatric patients found that ceralasertib plus olaparib was generally well tolerated with some instances of thrombocytopenia and neutropenia that were dose-limiting and has resulted in partial responses in two patients thus far (Gatz et al., 2023). All 17 ATR inhibitor drugs in clinical development are new chemical entities (NCEs). Currently, ceralasertib is the most advanced ATR inhibitor, with a Phase III clinical trial underway for non-small cell lung cancer (NSCLC).

**Key Considerations**

• Accurate biomarkers for ATR inhibitor sensitivity likely will involve functional readouts of the ATM pathway, which are more difficult and costly experiments than traditional biomarkers, making it hard to develop biomarkers to select appropriate patients for these agents

• ATR inhibitors may be effective in combination with various drug types, including antibody drug conjugates (ADCs), microenvironment-targeting drugs, drugs with non-overlapping side effect profiles, and DNA-damaging agents.

• Prior lines of treatment may impact tumor sensitivity to ATR inhibition via accumulation of additional alterations.

**Next Steps**

• ATR is a moderate priority drug target for further preclinical development at this time as reviewed by COACH.

• Preclinical research as well as patient tumor profiling to identify predictive biomarkers for ATR inhibitor response specific to pediatric tumors.

• Preclinical research should focus on sensitive tumor types and rational combination strategies identified from the results of ongoing adult clinical trials, including Antibody
drug conjugates (ADCs, drugs targeting tumor microenvironment, drugs with non-overlapping side effect profiles, and DNA-damaging agents.

**HDAC 1-3**

HDACs deacetylate histones to regulate gene expression, which can trigger anticancer activity. Mammals have 18 HDACs across four classes. Class I HDACs (HDAC1, 2, 3, and 8) are broadly expressed across cell and tumor types and are rarely altered in pediatric tumors. In pediatric cancer cell lines, genetic loss of HDAC3 severely impacts cell viability, while loss of HDAC1 and 2 have a more subtle effect on viability. Pharmacological inhibition of class I HDACs inhibits viability in osteosarcoma and rhabdomyosarcoma cell lines, and class I HDAC inhibitors have shown single-agent efficacy in several leukemia mouse models and some success in combination therapies in glioma mouse models. Across solid tumors, the Pediatric Preclinical Testing Consortium (PPTC) determined that two HDAC inhibitors—vorinostat and quisinostat—showed limited single-agent antitumor activity.

In tumor expression studies, expression of class I HDACs is negatively associated with survival in glioma, acute myeloid leukemia (AML), and EwS tumors. Notable clinical trials of HDAC1-3 inhibitors include: (1) a Phase I/II trial of entinostat at the recommended Phase II dose (RP2D) plus nivolumab in relapsed or refractory solid tumors; (2) a Phase I trial of mocetinostat plus vinorelbine for relapsed or refractory rhabdomyosarcoma; (3) a phase II/III clinical trial for pediatric high-grade glioma evaluating radiation therapy + either vorinostat or temozolomide or bevacizumab that showed no benefit for the addition of vorinostat (NCT01236560); (4) a randomized phase II trial evaluating the addition of vorinostat to MIBG for patients with relapsed/refractory neuroblastoma that showed a higher response rate with the addition of vorinostat, although PFS was not extended; and (5) Phase I trials of panobinostat plus gadolinium for newly diagnosed diffuse midline gliomas (DMGs). The entinostat combination trial determined that the RP2D was safe and tolerable, with only one adverse event—a grade three thrombocytopenia (van Tilburg et al., 2022). Mocetinostat plus vinorelbine demonstrated a clinical benefit rate of 86 percent, while convection-enhanced delivery (intratumoral) of panobinostat plus gadolinium resulted in an average progression-free survival (PFS) of 8 to 20 months (Jonsson Comprehensive Cancer Center, 2022; Szalontay, 2022; Zacharoulis et al., 2022). The HDAC inhibitor clinical development pipeline contains 40 drugs in development and 9 approved inhibitors. Notably, three HDAC inhibitors are FDA-approved to treat T-cell lymphoma (vorinostat, romidepsin, and belinostat). A 2015 FDA approval for multiple myeloma for panobinostat was withdrawn in 2021.

**Key Considerations**

- Pharmacokinetic (PK) studies that compare concentrations that are active *in vitro* to those that are achievable in the clinic are useful for transitioning HDAC1-3 inhibitors to clinical development though these studies are challenging to conduct.
- Preclinical research should use multiple, relevant biological replicates for more robust HDAC1-3 inhibitor findings.
• Each HDAC inhibitor is unique and has different side effects, although there appear to be class effects such as thrombocytopenia that are observed across multiple HDAC inhibitors. Cardiac toxicity, particularly arrhythmias, is also reported for some HDAC inhibitors.

• Most pediatric cancer cell lines are sensitive to HDAC inhibitors. Pediatric cancer in vivo models are generally not sensitive to single-agent HDAC1-3 inhibitors, though some level of activity has been observed for diffuse intrinsic pontine gliomas (DIPGs), fibrolamellar tumors, and hepatoblastomas.

• HDAC1-3 inhibitors may have synergistic therapeutic effects in combination with conventional chemotherapy, proteasome inhibitors, MEK inhibitors, and targeted immunotherapies.

• Rhabdomyosarcoma, DIPG, and neuroblastoma are all tumors for which clinical trials of HDAC1-3 inhibitors are ongoing or recently completed.

Next Steps

• HDAC1, 2, and 3 are medium to high priority targets for further preclinical development at this time as reviewed by COACH.

• Conduct further studies of HDAC biology to inform HDAC1-3 inhibitor in vitro and in vivo screens and identify promising inhibitor candidates and relevant tumor types.

• Invest in PK studies and better animal models to advance promising HDAC1-3 inhibitor candidates into clinical trials.

• Identify additional CNS-penetrant HDAC1-3 inhibitor treatment strategies.

• Use computational approaches to analyze in vitro and in vivo HDAC 1-3 inhibitor data to identify potential biomarkers for HDAC1-3 inhibitor sensitivity.

• Perform rational high throughput screening for potential combination therapies with the following drug classes: conventional chemotherapy, proteasome inhibitors, MEK inhibitors, and targeted immunotherapies.
Meeting Summary

Review of Meeting Objectives
Stacey Adam, PhD, Foundation for the National Institutes of Health (FNIH)

Dr. Stacey Adam welcomed meeting participants and reiterated that the goal of Convening Experts in Oncology to Address Children’s Health (COACH) is to provide expert recommendations regarding drug target prioritization and salient preclinical steps to either clarify the priority of a specific drug target, or prepare the drug target, for pediatric cancer clinical trials. If meeting participants identify a target as high priority, they will specify preclinical testing needed to advance agents to pediatric clinical trials or clarify existing clinical challenges. If meeting participants identify a target as low priority, they will determine whether additional preclinical data are required to advance the target to higher priority or declare it as not relevant for pediatric cancer indications. Meeting participants should also consider whether preclinical testing is needed to clarify targets currently inconclusive for relevance in pediatric cancer. Dr. Adam reminded meeting participants that COACH discussions should focus on drug targets and not agents; proprietary data for reviewed agents should not be discussed within COACH, unless private sector partners choose to disclose this information.

Pediatric Cancer Drug Target Data
Stacey Adam, PhD, Foundation for the National Institutes of Health (FNIH)

Dr. Adam presented data on relevant genetic alterations, transcriptomic expression, patient survival, and in vitro and in vivo dependency for three drug targets—phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), ataxia telangiectasia and Rad3-related protein (ATR), and histone deacetylase (HDAC) 1-3—as well as data on in vitro and in vivo drug sensitivity and clinical response rates for their respective therapeutics. The presence of alterations in a drug target within a given tumor type does not necessarily indicate that target would be an effective therapeutic target. In addition, a therapeutic formulated to inhibit a differentially expressed protein in a specific tumor type may not result in any significant therapeutic effect. Although there are limitations to in vitro dependency data, they can provide scientific rationale to support further preclinical assessments. However, in vitro dependency may not always reflect in vitro drug sensitivity. To address limitations associated with each of these data types, meeting participants considered these data in combination, identifying pediatric indications that may be sensitive to the target therapeutics.

Data types for each drug target were compiled from different databases, as well as scientific literature. Patient alteration data, compiled from cBioPortal and PedcBioPortal cohorts, included simple somatic mutations (SSMs), copy number variations (CNVs), and structural variations (SVs). Patient expression data were compiled from CCDI Molecular Targets Platform and XenaBrowser. In vitro dependency data were obtained from Dependency Map (DepMap) and presented using Chronos scores, a normalized metric of cell viability after gene deletion. A Chronos score of 0 indicates gene is non-essential, while score of -1 is comparable to the
median of all pan-essential genes. In vitro sensitivity data were represented with Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) scores (i.e., area under the curve [AUC] derived from eight-point dose-response curve ranging from 10µM to 610pM) from DepMap. PRISM score of 1 indicates complete lack of response at all concentrations, whereas score of 0 indicates complete loss of viability at all concentrations. In vivo dependency and drug sensitivity data were aggregated from relevant scientific literature.

Research landscape data included federal grant spending, publications, and general commercial activity relevant to each target. Federal grant data obtained from the National Institutes of Health (NIH) were further classified by subtopics and federal agency administrators. Publication data were analyzed using PubTator. Commercial activity was summarized from Citeline’s PharmaProjects and Trialtrove databases.

PIK3CA
Stacey Adam, PhD, Foundation for the National Institutes of Health

Overview
Phosphatidylinositol-4,5-biphosphate 3-kinase, catalytic subunit alpha (PIK3CA) encodes the p110α subunit of a class I phosphatidylinositol 3-kinase (PI3K), a component of the PI3K/AKT serine/threonine kinase (AKT) signaling pathway. This signaling pathway has numerous downstream effects on cell metabolism and growth through direct regulation of nutrient transporters and metabolic enzymes, as well as control of transcription factors that regulate expression levels of key components of metabolic pathways.

PI3K signaling is activated by various receptor tyrosine kinases (RTKs) that transduce signals from extracellular growth factors (e.g., epidermal growth factor [EGF], platelet-derived growth factor [PDGF], and insulin-like growth factor [IGF]). PI3K phosphorylates phosphatidylinositol 4,5-biphosphate (PIP2) to form phosphatidylinositol (3,4,5)-triphosphate (PIP3, a PI3K secondary messenger), and phosphatase and tensin homolog (PTEN) can reverse this phosphorylated state. As PIP3 accumulates, it forms docking sites that recruit effector proteins, including AKT, which can then phosphorylate several downstream substrates to influence cell growth, proliferation, and survival.

Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival
Gene alterations in PI3K/AKT signaling components in cancers include mutations in PIK3CA, loss-of-function (LOF) mutations and deletions in PTEN, and amplification and activation of specific PI3K-activating RTKs. While uncommon in pediatric cancers in general, these mutations are observed in an appreciable number of adult and pediatric central nervous system (CNS) tumors. High PIK3CA expression is associated with improved survival in a cBioPortal cohort of glioma patients, and alterations in PI3K class I genes in glioblastoma patients are associated with improved outcomes (Yan et al., 2020). PI3KCA and related mutation rates in pediatric cancers are highest in gliomas (including diffuse intrinsic pontine gliomas [DIPGs] and non-brain stem high-grade gliomas [HGGs]) and have also been reported for malignant peripheral nerve sheath tumors (MPNSTs) (Brohl et al., 2017).
In a dataset of over 5,800 pediatric tumors, 61 had PIK3CA mutations, including 4 of 67 non-brainstem HGGs and 10 of 57 DIPGs. Based on analysis of 6,477 samples across 12 studies, PIK3CA is mutated in 36 percent of adult breast cancer patients, with mutations frequently located in exons 4, 9, and 20. Notably, PIK3CA mutations are located within the same hotspots across adult and pediatric tumors. Unlike many other genes mutated in cancer—which are often not shared between adults and pediatric patients—similar mutational hotspots across adult and pediatric tumors indicate the potential translatability of adult therapies to pediatric patients. However, PIK3CA mutant allele frequencies in pediatric tumors are often less than 20 percent, suggesting that these mutations may be subclonal and possibly non-causative.

**Dependency and Drug Sensitivity**

While PIK3CA is poorly expressed in most pediatric cancer cell lines—especially compared to adult cancer cell lines—its expression is still required for cell viability in all pediatric tumor types. One notable exception is the hepatoblastoma cell line HUH6, which has high PIK3CA expression. Genetic LOF results in substantially reduced viability in all tumor types, especially Ewing sarcomas (EwSs), malignant rhabdoid tumors (MRTs), and rhabdomyosarcomas (RMSs); these results are in agreement with the well-established role of PI3K/AKT signaling in RMS (Renshaw et al., 2013). Despite widespread sensitivity of tumor cell lines to PI3KCA LOF mutations, only MRT cell lines were significantly affected by alpelisib (a PIK3CA inhibitor) in the PRISM drug screen dataset.

Generally, PI3K inhibitors have limited single-agent activity in pediatric cancer mouse models of leukemia, but efficacy studies are currently underway to assess combination therapies targeting related signaling pathways, such as Janus kinase (JAK). In an acute lymphocytic leukemia (ALL) xenograft mouse model, the PI3K inhibitor, BEZ235, improved survival, and in combination with ruxolitinib (a JAK inhibitor) led to complete regression in bone marrow and spleen (Y. Cheng et al., 2016). Testing of another PI3K inhibitor, SAR245408, in six cell line xenograft models resulted in progressive disease (PD) in all six models, though event-free survival (EFS) increased in two of six models (Reynolds et al., 2013). While single agent treatment of a T-cell ALL (T-ALL) xenograft mouse model with the PI3K inhibitor, GDC-0032, did not impact T-ALL burden, dual targeting with nifuroxazide (a STAT3 inhibitor) repressed tumor growth (Yuzugullu et al., 2016). In addition, a combination of PI3K inhibitor, BYL719, plus mitogen-activated protein kinase (MAPK)/extracellular signal-regulated (ERK) kinase (MEK) inhibitor MEK162 reduced overall tumor burden in THP-1 cell line-derived xenograft (CDX) model of acute myeloid leukemia (AML) (Gritsman et al., 2014). PI3K inhibitor GDC-0980 plus venetoclax (a B-cell lymphoma 2 [BCL-2] inhibitor) reduced AML burden, did not impact normal hematopoietic progenitors, and prolonged survival or diminished growth in systemic mouse xenografts and two patient-derived (PDX) xenografts (Rahmani et al., 2018).

PI3K inhibitors have also shown modest single-agent activity in glioma mouse models but have potential for success in synergistic treatment combinations. Single-agent activity of the PI3K inhibitor paxalisib was poor in a pediatric mouse model of DIPG, although the agent had synergistic effects with mirdametinib, a MEK inhibitor (He et al., 2021). However, in a subcutaneous in vivo model of DIPG, combination treatment of the PI3K inhibitor, ZSTK474, and
MEK inhibitor (trametinib) resulted in low activity (Chang et al., 2019). PI3K inhibitors also have mild activity in glioblastoma and other glioma mouse models, but this activity may be improved through drug targeting of potential resistance pathways. The PI3K inhibitor, SAR245408, (pilaralisib) prolonged EFS in four of five glioblastoma xenograft mouse models, but none showed complete response (CR) (Reynolds et al., 2013). However, in glioma stem-like cell (GSC) intracranial xenografts (GSC 7-11), PI3K inhibition combined with an Aurora A inhibitor targeting a potential resistance pathway reduced tumor burden and improved survival (Li et al., 2019). In addition, the PI3K inhibitor, PIK-90, prevented tumor growth in glioblastoma multiforme (GBM) xenografts (GBM43) when administered in combination with a cyclin-dependent kinase (CDK) 1/2 inhibitor (C. K. Cheng et al., 2012).

Pediatric Preclinical Testing Consortium (PPTC) studies show limited activity of PI3K inhibitors in pediatric solid tumors. Pilaralisib had mild activity that resulted in, at best, prolonged EFS, followed by PD in 2 of 6 ALL mouse models, 0 ependymoma models, 3 of 5 EwS models, 4 of 5 glioma models, half of all tested medulloblastoma models, and all RMS and Wilms tumor models tested. In addition, copanlisib treatment resulted in prolonged EFS, but no tumor regression, in 5 of 6 osteosarcoma models tested (Harrison et al., 2023). Together, these results suggest that single-agent PI3K treatment regimens result in resistance accumulation followed by cancer progression.

PIK3CA knockout (KO) preclinical studies have identified potential for toxicities in blood glucose homeostasis and reproductive organ functions. PIK3CA KO mice are non-viable with fully penetrant lethality during late embryogenesis, despite no detectable defects in organogenesis. At embryonic day 9.5, cardiac function is evident, but the developing heart is smaller than wildtype mice. Lethality in these mice is due to eventual hemorrhaging in the forehead and snout (Bi et al., 1999; Lelievre et al., 2005). These vessels are dilated and exhibit reduced branching during morphogenesis (Lelievre et al., 2005). Heterozygous PIK3CA mice are viable and fertile but experience blunted insulin receptor signaling, resulting in reduced somatic growth, hyperinsulinemia, glucose intolerance, hyperphagia, and increased adiposity (Foukas et al., 2006). Postnatally, heterozygous mice develop mild anemia without impacts on hematopoietic stem cell (HSC) renewal (Gritsman et al., 2014), which is consistent with observations of increased blood glucose and compensatory insulin release in wildtype mice after PI3K inhibition via alpelisib, buparlisib, and taselisib (Hopkins et al., 2018). In addition, more than half of adult patients taking alpelisib report hyperglycemia (Nunnery & Mayer, 2019).

PI3K inhibitors likely do not induce genetic damage, but they impact both male and female reproductive systems. Rat micronucleus tests of copanlisib and alpelisib indicate that these PI3K inhibitors do not induce genetic damage (ALIQOPA (Copanlisib) for Injection, for Intravenous Use, 2017; PIQRAY (Alpelisib) Tablets, for Oral Use, 2019). However, repeat-dose toxicity studies in rats and dogs showed perturbations in the testes (e.g., germinal epithelial degeneration, decreased weight, tubular atrophy), epididymides, and prostate (e.g., reduced secretion and weight); and in female mice, repeat-dosing resulted in abnormalities in the ovaries (e.g.,
hemorrhage, hemorrhagic cysts, decreased weight), uterus (e.g., atrophy, decreased weight), vagina (e.g., mononuclear infiltration), and estrus cycle (i.e., reduction of females in estrus).

**Clinical Trial Development**
A Phase I/II clinical trial of copanlisib is ongoing, recruiting Phase II patients from 6 months to 21 years of age with relapsed or refractory solid tumors, including EwS, neuroblastoma, osteosarcoma, and rhabdomyosarcoma. This trial is assessing efficacy (e.g., objective response rate [ORR], disease control rate [DCR], and progression-free survival [PFS]) and safety (e.g., adverse event, dose-limiting toxicity, and maximum tolerated dose [MTD]) in patients diagnosed with relapsed or refractory solid tumors or lymphoma after failure of first-line treatment (Phase I) and in patients diagnosed with EwS, neuroblastoma, osteosarcoma, and rhabdomyosarcoma (Phase II). In Phase I, 22 of 23 patients had no objective response (OR); 3 of 23 had stable disease (SD), and 19 of 23 had PD. Reported toxicities were as expected, with treatment emergent adverse events (TEAEs) of hyperglycemia (70% of patients), nausea (61%), and decreased white blood cell count (52%). Additional toxicities reported in other clinical trials include rash, diarrhea, and low-grade stomatitis (Rugo et al., 2020). Notably, observed pharmacokinetic (PK) and pharmacodynamics in pediatric patients were similar to those in adult patients (Bayer, 2023; Macy et al., 2022).

**Research and Development Landscape**
Approximately 50 percent of PIK3CA-related grants are administered by the National Cancer Institute (NCI). Current literature indicates some interest in PIK3CA as a potential target in oncology, with 59 percent of PIK3CA articles being oncology-related. However, there is a paucity of PIK3CA articles on pediatric oncology. Glioma is the most frequently mentioned pediatric oncology indication in PIK3CA articles, followed by Hodgkin’s lymphoma (HL), osteosarcoma, neuroblastoma, non-Hodgkin’s lymphoma (nHL), and ALL. Hepatoblastoma and clear cell sarcoma of the kidney (CCSK) are only referenced in a single article each, and MRT and pineoblastoma are not referenced in any PIK3CA-related research articles.

PIK3CA as an oncology drug target has considerable competition compared to other oncology targets, with 33 drugs in active development—most of which (97.1%) are new chemical entities (NCEs), indicating a high level of innovation—and eleven pediatric clinical trials are ongoing or planned. Both copanlisib (Bayer) and alpelisib (Novartis) are FDA-approved. Alpelisib is the first FDA-approved drug for PIK3CA-related overgrowth spectrum (PROS) and is approved for patients two years and above who require systemic therapy. Retrospective chart review of PROS patients through the EPIK-P1 study identified 27 percent confirmed responses at week 24 of treatment, 74 percent of patients with target lesion volume reduction, and 0 percent of patients with disease progression (Novartis Pharmaceuticals, 2022). Notably, target accruals for trials including pediatric patients are high compared to pediatric trials for other oncology targets, indicating relatively high-cost trials.

**Additional Considerations for Preclinical and Clinical Development**
*Facilitated by Ron Bernardi, MD, PhD, Genentech*
After the conclusion of Dr. Adam’s PIK3CA presentation, Dr. Ron Bernardi facilitated a discussion on toxicity concerns, complexity of CNS tumors and blood-brain barrier (BBB) penetration, rational treatment combinations, and strategies for patient selection.

**Toxicity Concerns**

Overall, meeting participants expressed concern about PI3K therapeutic toxicities and whether they can be mitigated in pediatric patients. Overall concerns about PI3K toxicities and narrow therapeutic window of PI3K inhibitors have limited development of pediatric preclinical research plans. Dr. Susanne Gatz explained that waiting for results from adult clinical trials may provide insight into combinations with low toxicity that may also have low toxicity in pediatric patients. In addition, although PI3K inhibitors have shown preclinical success in certain CNS tumor models, CNS-penetrant PI3K inhibitors have neuropsychiatric toxicities, which may or may not be an on-target effect. Therefore, further mechanistic research is required to understand these PI3K inhibitor toxicities before proceeding with CNS-penetrant inhibitors in pediatric indications.

**Central Nervous System Tumor Complexity and Crossing the Blood-Brain Barrier**

Only some PI3K inhibitors can penetrate the BBB, and preclinical in vivo models of PI3K mutant tumors may not sufficiently reflect the overall complexity in pediatric CNS tumors. Pediatric CNS tumors are difficult to model because many PI3K mutations are subclonal and may not be cancer driving mutations. While some preclinical models have PIK3CA mutations, these are CDXs that are subsequently intracranially injected with PI3K inhibitors, which may not reflect therapeutic approaches in the clinic.

**Rational Treatment Combinations**

Successful adult PI3K inhibitor combinations, including with endocrine and CDK4/6 inhibitors, may be irrelevant to pediatric cancers. Other dual PI3K/mTOR inhibitors may provide synergistic treatment effects, but this may be accompanied by synergistic toxicities. Dr. Malcolm Smith cautioned against pursuing a broad preclinical combination research plan to screen for effective PI3K inhibitor treatment combinations; however, some rational combinations may provide predicted synergy that should be investigated further. For example, inhibition of tumor escape pathways may improve treatment responses without intolerable toxicities.

With alpelisib approved to treat PROS, review of existing clinical data as well as post-marketing data may provide further insights relevant to treatment of pediatric cancer patients. In addition, patients with other rare syndromes characterized by activating mutations in the PI3K signaling pathway (e.g., activating p110δ syndrome 1 and 2, megalencephaly-capillary malformation syndrome, megalencephaly-polydactyly-polymicrogyria-hydrocephalus, PTEN hamartoma tumor syndrome, Proteus syndrome, hypoglycemia with segmental overgrowth, and tuberous sclerosis complex) may also benefit from alpelisib and other PI3K pathway inhibitors (e.g., everolimus, rapamycin, miransertib, nemiralisib), especially those with PTEN hamartoma tumor syndrome (Boston Children’s Hospital, n.d.; Madsen et al., 2018; National
Cancer Institute, n.d.). Data from various clinical trials of PI3K pathway inhibitors in these PROS-related syndromes may provide additional insights related to pediatric cancer patients.

**Patient Selection**

Initial pediatric PI3K inhibitor clinical trials did not select patients based on specific biomarkers; instead, these trials enrolled only patients with PIK3CA alterations. However, preclinical research and results from PROS patients treated with alpelisib suggest that patients with alterations in the PI3K pathway, not just PIK3CA, may respond to PI3K inhibitors.

**Next Steps**

In summary, PIK3CA is a low priority target because of high inhibitor toxicities, lack of identified synergistic treatment combinations, and unclear relevant pediatric populations. Results from adult PI3K inhibitor clinical trials may provide insight into potentially relevant pediatric patient populations as well as rational treatment combinations with synergistic therapeutic effects and without synergistic toxicities.

**ATR**

*Stacey Adam, PhD, Foundation for the National Institutes of Health*

**Overview**

ATR is a central player in the DNA damage response pathway, which can affect cellular checkpoints. ATR is a kinase, activated by DNA damage and replication stress, that transduces checkpoint signaling pathways to regulate replication and cell cycle transitions. ATR detects DNA damage and replication stress in part by recognizing single-stranded DNA (ssDNA) coated with replication protein A (RPA) during S-phase. RPA binds ATR-interacting protein (ATRIP), which then recruits ATR. ATR phosphorylates various targets—including Werner syndrome ATP-dependent helicase (WRN), switch/sucrose non-fermentable (SWI/SNF) related, matrix associated, actin dependent regulator of chromatin, subfamily a like 1 (SMARCAL1), and Fanconi anemia complementation group I (FANCI)—that slow origin firing, induce cell cycle arrest, and stabilize and restart stalled replication forks.

**Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival**

While mutations in ATR are uncommon, more common mutations in ataxia telangiectasia mutated (ATM), and related genes, may render tumor cells sensitive to ATR inhibition, resulting in synthetic lethality (Waskiewicz et al., 2021). Loss of ATM in preclinical models of prostate cancer sensitizes these tumors to ATR inhibition (Rafiei et al., 2020), and ATR inhibitors demonstrate synthetic lethality in chronic lymphocytic leukemia (CLL) cell lines with defects in ATM or P53 (Kwok et al., 2016; Rafiei et al., 2020). In addition, early clinical studies have detected responses to ATR inhibition in advanced solid tumors with ATM loss (Mahdi et al., 2021; Yap et al., 2021). Other perturbations that sensitize tumors to ATR inhibition include expression of DNA transposase PiggyBac transposable element-derived 5 (PGBD5) and alternative lengthening of telomeres (Flynn et al., 2015; Henssen et al., 2017). Despite ATR’s role in maintaining genomic integrity, mutations in ATR are not considered a strong driver of
carcinogenesis. ATR is expressed ubiquitously across hematologic and solid tumors, but its expression is not correlated with any survival outcomes.

**Dependency and Drug Sensitivity**

Consistent with patient tumor expression data, ATR is broadly expressed at similar levels in pediatric cancer cell lines, with ALL and nHL cell lines having slightly higher ATR expression than other cell lines. Generally, pediatric cancer cell lines are sensitive to loss of ATR expression, with MPNST, osteosarcoma, and retinoblastoma lines being the most sensitive to ATR loss. However, these same cancer cell lines are far less responsive to pharmacologic inhibition of ATR. Pediatric cell lines treated with the ATR inhibitor VE-821 showed minimal responses, but treatment with the ATR inhibitor VE-822 resulted in stronger responses in EwS and neuroblastoma cell lines.

Several ATR inhibitors have exhibited strong activity as single agents, as well as in combination regimens, in leukemia mouse models. Daily dosing of B-cell ALL (B-ALL) models with VE-822 slowed tumor progression by impairing nucleotide biosynthesis. In combination with other nucleotide biosynthesis inhibitors (i.e., ribonucleotide reductase and deoxycytidine kinase inhibitors), VE-822 induced sustained remission in B-ALL models (Le et al., 2017). In a Ras-driven B-ALL model, the ATR inhibitor AZ20 showed single-agent activity and, when combined with MEK inhibition, induced complete remission (CR) (Chu et al., 2018). AZ20 also showed strong single-agent activity in an a mixed-lineage leukemia (MLL)-rearrangement mouse model but did not induce remission. Notably, initiating AZ20 treatment at the time of tumor engraftment prevented tumor expansion (Morgado-Palacin et al., 2016). However, the ATR inhibitor AZD6738 did not induce treatment responses in T-ALL PDX models without BRCA2 mutations (Pouliot et al., 2019), and BRCA2 haploinsufficiency induces hypersensitivity to ATR inhibition.

Similar to leukemias, the efficacy of ATR inhibition in solid tumor models is poorly understood, but initial responses suggest ATR may be useful for the treatment of sarcomas and gliomas. The Pediatric Preclinical Testing Program (PPTP) extensively tested the ATR inhibitor M6620 (i.e., VX-970) as a single agent and in combination with chemotherapy. M6620 showed limited single-agent activity in all xenograft models, resulting in PD, and M6620 plus cisplatin was significantly superior to cisplatin alone in only 4 of 24 models tested. Notably, the Wilms tumor model KT-5 exhibited PD with single-agent M6620 but entered complete remission in M6620 plus cisplatin treatment settings (Kumasheva et al., 2018). Two ATR inhibitors—AZD6738 and BAY-1895344—exhibited strong single-agent activities in alveolar RMA tumors expressing paired box gene 3 (PAX3) and forkhead box protein O1 (FOXO1). Another ATR inhibitor—RP-3500—showed significant single-agent activity in gastric and colon cancer CDX models, even at low doses that minimize toxicities. In combination with a low-dose PARP inhibitor in a short-term intermittent schedule, RP-3500 resulted in sustained anti-tumor effects, and RP-3500 is currently in Phase I/IIa clinical trials in adult patients with advanced solid tumors (Roulston et al., 2022; Zimmermann et al., 2022). In combination with a poly [ADP-ribose] polymerase 1 (PARP1) inhibitor, ATR inhibition triggered complete regression of RMS PDXs (Dorado García et al., 2022).
ATR is required during embryogenesis and for tissue homeostasis. Thus, ATR KO mice are non-viable and die during early embryogenesis (Brown & Baltimore, 2000). In adult mice, induced loss of ATR resulted in defects in tissue homeostasis and age-related phenotypes associated with reductions in tissue-specific stem and progenitor cells and lack of tissue renewal (e.g., hair graying, alopecia, kyphosis, osteoporosis, thymic involution, fibrosis) (Ruzankina et al., 2007). Combined loss of ATR and P53 exacerbated these tissue degeneration phenotypes—leading to localized inflammation, deterioration of the intestinal epithelium, and synthetic lethality in adults (Ruzankina et al., 2009)—whereas hypomorphic expression of ATR resulted in few defects in bone marrow cellularity and epithelium deterioration (Schoppy et al., 2012).

Combination treatment with 20 mg/kg of M6620 plus varied cisplatin doses up to 5 mg/kg was well tolerated in mouse models, with temporary weight loss that recovered during the treatment period (Kurmasheva et al., 2018). Neither AZD6738 nor BAY-1895344 significantly affected body weight or histopathology of major organs (Dorado García et al., 2022). However, in a mouse RMS model, BAY-1895344 resulted in reduced erythrocyte counts, consistent with a first-in-human study reporting hematologic TEAEs (Dorado García et al., 2022; Yap et al., 2021).

Clinical Trial Development
ATR inhibitor studies are currently in Phase I/II and Phase II, with no ongoing or planned pediatric Phase III trials. The Dana Farber Cancer Institute is sponsoring a Phase II study of the ATR inhibitor ceralasertib plus olaparib, a PARP inhibitor, in recurrent osteosarcoma patients between the ages of 12 and 40 years old (Dana-Farber Cancer Institute, 2022). This study will also use pre- and post-treatment tumor samples for further biomarker studies and to derive PDX models. An NCI-sponsored Phase I/II study of the ATR inhibitor elumisertib will evaluate best, complete, and partial responses (PRs), as well as dose-limiting toxicity in patients between the ages of 12 months and 30 years old with solid recurrent or refractory tumors, including EwS and alveolar RMS (National Cancer Institute (NCI), 2023a). Both studies are currently recruiting, and no results are available. A European Phase I/II proof-of-concept study of ceralasertib plus olaparib has enrolled 18 pediatric patients, and five patients encountered dose-limiting thrombocytopenia or neutropenia. One pineoblastoma patient exhibited partial response, and a neuroblastoma patient converted from stable disease to partial response after treatment cycle nine. This study is ongoing and includes biomarker analysis to identify factors associated with efficacy of ceralasertib plus olaparib.

Research and Development Landscape
More than half of all ATR-related grants are administered by NCI, and 49 percent of ATR-related publications focus on oncology. However, less than 1 percent of these ATR publications are relevant for pediatric oncology. Of the select pediatric oncology indications analyzed, glioma was referenced most frequently in ATR-related articles (25 articles), while EwS, osteosarcoma, and AML are each referenced in more than five articles. Wilms tumor, nHL, synovial sarcoma, and hepatoblastoma are each referenced in only 1 ATR-related article; ependymoma, MRT, pineoblastoma, malignant nerve sheath tumor (MNST), and CCSK are not referenced in any ATR articles. There are no approved ATR inhibitors, and all ATR drugs in development are NCEs, so
the market likely will not be saturated with reformulated drugs. The most advanced ATR inhibitor—ceralasertib—is currently in Phase III trials for non-small cell lung cancer (NSCLC). So far, ATR-targeting drugs have demonstrated a lower failure rate than other oncology drug targets, and overall clinical feasibility for this target is high.

**Additional Considerations for Preclinical and Clinical Development**

*Facilitated by Susanne Gatz, Dr. Med., University of Birmingham, and Michael Ortiz, MD, Memorial Sloan Kettering Cancer Center*

**Biomarker Identification**

The identification of predictive biomarkers for ATR inhibitor response requires additional preclinical research as well as patient tumor profiling. Currently, companies sponsoring trials of ATR inhibitors in adults each use different selection criteria and biomarkers, including types of ATM mutations; however, these adult biomarkers are very rare in pediatric patients. Identification of biomarkers in pediatric patients will enable more targeted and informed preclinical research. In addition, tumors likely sensitive to ATR inhibitors are driven by aberrant transcription factor fusions (e.g., SS18-SSX fusion in synovial sarcoma, PAX3-FOXO1 fusion in alveolar rhabdomyosarcoma) and likely by different ways of ATM activity reduction or compromise of HRD in pediatric cancers (Dorado García et al., 2022; Jones et al., 2017). Therefore, additional preclinical research is needed to identify relevant molecular mechanisms indicative of ATR inhibitor sensitivity. Moreover, the accurate identification of susceptible tumors in patients may require multiple biomarkers comprising a more complex molecular signature that includes ATM and other protein expression data and functional readouts of the ATM pathway. As research groups continue to collect patient tumor samples, researchers should carefully plan molecular profiling strategies around well-informed hypotheses of patients in ATR inhibitor trials (i.e., with information on response to treatment available to enable retrospective correlation).

**Combination Therapies**

Meeting participants strongly advocated for testing various combination therapeutic strategies with ATR inhibitors. However, candidate ATR inhibitors should demonstrate single-agent activity in the tumor type of interest before proceeding with any combination screening for synergistic drugs. Dr. Michelle Monje explained that clinical trials must be designed with enough sensitivity to detect single-agent activity. In addition, results from ongoing clinical trials may provide insight into other combination treatment options.

Any preclinical screening strategy should first prioritize combinations most likely to succeed; for example, some cytotoxic drugs may work well with ATR inhibitors, but treatment sequence and dosing is extremely important to maximize therapeutic effect and minimize synergistic toxicities. Meeting participants suggested the following potential ATR inhibitor combination strategies for preclinical pursuit:
• **Antibody drug conjugates (ADCs):** Because ADCs are designed for high specificity, ADCs with payloads likely to synergize with ATR inhibitors could result in high treatment efficacy without synergistic toxicities.

• **Drugs targeting tumor microenvironment:** ATR inhibitors address tumor cell intrinsic factors, while drugs targeting the tumor microenvironment may address other, non-overlapping drivers of cancer.

• **Drugs with non-overlapping side effect profiles:** Selecting drug combinations without overlapping side effect profiles helps minimize the risks for synergistic toxicities.

• **DNA-damaging agents:** Use of ATR inhibitors with DNA-damaging agents may enable a lower therapeutic dose of these cytotoxic drugs.

These combination strategies are well-informed but should be assessed in an unbiased manner.

**Treatment of Relapsed Versus Untreated Tumors**
Typically, clinical trials administer drugs in patients as second-, third-, or even fourth-line treatments, and the prevailing assumption is that if a relapsed or recurrent cancer patient responds to a therapeutic after multiple prior treatment regimens, that patient would have also responded if this therapeutic was administered as a first-line treatment. However, Dr. Michael Ortiz noted that, in the preclinical space, PDXs from the same patient, but generated from tissues collected at different time points relative to treatment lines, respond differently to the same ATR inhibitor. These differential responses suggest that initial treatments result in accumulation of additional alterations that sensitize these tumors to ATR inhibition.

**Next Steps**
ATR is a moderate priority drug target. Additional preclinical studies may help to advance ATR inhibitors into the pediatric clinical space; however, results of ongoing clinical studies, which will be released within the next year, are critical for identifying sensitive tumor types and potential combination treatment strategies that maximize therapeutic effect while minimizing synergistic toxicities.

**HDAC1-3**
*Stacey Adam, PhD, Foundation for the National Institutes of Health*

**Overview**
HDACs mainly target histones, regulating downstream gene expression via deacetylation of histones. This deacetylation is reversible by histone acetyltransferases (HATs), and disruption of this acetylation balance can result in aberrant expression of gene networks. HDAC-mediated deacetylation induces upregulation of oncogenes and activation of pro-cancer transcription factors, including specificity protein 1 (SP1) and CCAAT/enhancer-binding protein alpha (CEBPA). HDAC1-3 can also deacetylate non-histone proteins—most of which are transcription factors. Deacetylation of transcription factors can affect their stability, interactions, localization, and molecular functions. HDAC3 has more diverse non-histone targets than HDAC1 and 2.
Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival

Class I HDACs (i.e., HDAC1, 2, 3, and 8) are highly expressed in most tumor cells, but mutations in these genes are uncommon in pediatric cancers. Notably, HDAC2 is highly expressed in pediatric medulloblastomas, while expression of class I HDACs is correlated with poor survival in glioma, AML, and EwS patients (Park et al., 2003). Studies of in vitro pediatric cancer cell lines have not identified significant trends in class I HDAC expression levels across indications, but one study reported high expression of class I HDACs in EwS cell lines (Schmidt et al., 2021).

Dependency and Drug Sensitivity

Loss of HDAC3 greatly impacts cancer cell viability in vitro, while loss of either HDAC1 or 2 often induces more subtle effects. RMS cell lines are especially sensitive to loss of class I HDAC expression with loss of HDAC1, 2, or 3 having significant effects on viability. In contrast, EwS and retinoblastoma cell lines are resistant to loss of class I HDAC expression. Studies in osteosarcoma lines have shown that combined loss of HDAC1 and HDAC2 is required for loss of viability (McGuire et al., 2020), and osteosarcoma and rhabdomyosarcoma cell lines are sensitive to class I HDAC inhibitors (Murahari et al., 2017). Additional in vitro studies have shown that panobinostat can inhibit RMS cell line growth by repressing c-Myc (Hedrick et al., 2015). However, in vitro studies using vorinostat indicate that achievable doses in humans may be insufficient to affect tumor viability. Vorinostat is commonly used at 2.5 to 10 µM in vitro, with an IC50 greater than 1 µM, but the maximum achievable concentration of vorinostat in humans is between 1 to 2 µM (Badros et al., 2009; Butler et al., 2000; Kelly et al., 2005; Wilson et al., 2013; Yin et al., 2007). Vorinostat also has a very short half-life (between 1.5 and 2 hours), meaning therapeutic dosing is only maintained for a few hours after treatment (Ramalingam et al., 2007; Rubin et al., 2006). Notably, other HDAC inhibitors also show in vitro efficacy at doses that are not achievable in the clinic.

In vivo preclinical studies have shown efficacy of class I HDAC inhibitors in multiple leukemia models. Depletion of HDAC3 in MLL-AF9 fusion AML cells in vivo slowed recurrence and enhanced sensitivity to conventional chemotherapy. Similar results were achieved using the HDAC3-selective inhibitor, RGFP966 (Long et al., 2017). In addition, silencing of HDAC1 in K562 CDXs reduced tumor burden, while entinostat exhibited single-agent efficacy in a core-binding factor subunit beta (CBFβ)-smooth muscle myosin heavy chain (SMMHC) fusion AML model (Huang et al., 2014; Richter et al., 2019). The class I HDAC inhibitor, givinostat, was tested in 9 PDX models of T-ALL, resulting in 5 responder models and 4 poor or partial responder models. Models that responded had CR in blood and spleen CD7+ cells, but there were no long-term survivors (Pinazza et al., 2016). In addition, the dual HDAC1/2 inhibitor, Merck60, reduced leukemic burden in a B-ALL model, but this did not result in CR (Stubbs et al., 2015). PPTC tested vorinostat and quisinostat in 8 ALL xenografts, resulting in no improved EFS for vorinostat and 25 percent CRs with quisinostat (Carol et al., 2014; Keshelava et al., 2009).

To date, combination therapies in preclinical models have demonstrated efficacy in glioma but have limited efficacy in RMS models. The brain-penetrant HDAC inhibitor, RG2833, showed on-target activity in an orthotopic DIPG mouse model, as well as strong synergistic activity with
paxalisib (a PI3K inhibitor) in clinically relevant doses (Barnett et al., 2022). Another HDAC inhibitor, RGFP966, showed moderate activity against GBM GCSs as a single agent and resulted in SD when combined with the bromodomain-containing protein 4 (BRD4) inhibitor JQ1 (an IL6 and STAT3 inhibitor) (Wang et al., 2020). In addition, depletion of HDAC1 extends survival in PDX and mouse models of GBMs (Cascio et al., 2021). In contrast, combination treatment with vincristine plus entinostat did not consistently improve treatment responses in 8 alveolar RMS models, compared to single-agent administration of either drug (Bharathy et al., 2018). Combination of entinostat plus standard of care chemotherapy also did not improve tumor responses in four PDXs compared to chemotherapy alone (Kurmasheva et al., 2019).

PPTC performed studies using vorinostat and quisinostat as single agents across solid tumor in vivo models. Although quisinostat had broad impacts on viability across pediatric cancer cell lines in vitro, this drug had consistent anti-tumor activity in only ependymoma (prolonged EFS with PD in one model), glioma (prolonged EFS with PD in 2 of 4 models), and medulloblastoma (prolonged EFS with PD in 1 of 2 models). Single-agent activity for vorinostat was poor, with no ORs observed across tested xenograft models (Carol et al., 2014; Keshelava et al., 2009).

While broad HDAC deletion is embryonic lethal, pharmacological inhibition is associated only with thyroid and bone abnormalities. In mice, deletion of HDAC1 is fatal by embryonic day 9, while deletion of HDAC2 results in perinatal fatality due to cardiac defects. However, cardiac-specific deletion of HDAC1 or HDAC2 did not cause a detectable phenotype, but cardiac-specific deletion of both genes resulted in neonatal lethality, with cardiac arrhythmias and dilated cardiomyopathy (Montgomery et al., 2007). Conditional deletion of both HDAC1 and HDAC2 in the female germline resulted in arrest of follicular development at the secondary follicle stage, perturbation of the transcriptome, and global reduction of transcription, despite increased histone acetylation (Ma et al., 2012). A 24-week repeat-dose canine toxicity study of belinostat found reduced testis/epididymide weights and delayed testicular maturation (BELEODAQ (Belinostat) for Injection, for Intravenous Administration, 2014). In addition, oral administration of panobinostat in rats and dogs decreased triiodothyronine, tetraiodothyronine, and thyroid stimulating hormone. Rodents administered panobinostat also developed hyperostosis, plasmacytosis, increased number of granulocytic cells, and abnormal cytoplasm granulation (FARYDAK (Panobinostat) Capsules, for Oral Use, 2015). Notably, structurally diverse HDAC inhibitors caused delayed but persistent increases in corrected QT interval (QTc) in canines (Spence et al., 2016).

**Clinical Trial Development**

Consistent with HDAC inhibitor studies in canines, clinical studies of HDAC inhibitors have raised concerns about cardiac abnormalities, most commonly QTc prolongation. Other cardiac events observed in HDAC inhibitor trials include atrial fibrillation, ventricular tachycardia, torsade de pointes, and ventricular fibrillation. Weekly intravenous administration of 15mg/m² panobinostat (80 percent of the maximum tolerable dose in adults) in pediatric patients with refractory solid tumors triggered grade 2 prolonged QTcs in 2 of 6 patients, with one cardiac dose-limiting toxicity (DLT) and T wave changes in inferior leads (Wood et al., 2018). In adults with lymphomas and solid tumors, intravenous panobinostat administered at escalating doses
on a daily or weekly schedule triggered a 13-beat episode of torsades de pointes and prolonged QTc in one patient of eight at the 20mg/m² dose.

Escalating doses of oral quisinostat—between 6 and 12 mg on days 1, 3, and 5 each week—plus 1.3 mg/m² bortezomib and 20 mg oral dexamethasone in cycles of 21 or 35 days did not result in DLTs in groups administered 6 to 8 mg of quisinostat. However, higher doses resulted in one grade 4 cardiac arrest in the 10 mg cohort, one grade 3 QTc prolongation in the 12 mg cohort, and one grade 3 atrial fibrillation in the 12 mg cohort (Moreau et al., 2016). In another escalating dose study of oral quisinostat—with continuous or intermittent treatment schedules in adults with solid tumors—intermittent treatment triggered cardiac DLTs (prolonged QTc and hypertension) in 2 of 26 patients; elevated troponin was also observed in the intermittent treatment group (Venugopal et al., 2013). A case study of a 49 year-old male receiving vorinostat for recurrent or persistent cutaneous T-cell lymphoma reported development of prolonged QTc and a pulseless polymorphic ventricular tachycardia that required resuscitation (Lynch et al., 2012).

HDAC1-3 inhibitors are being tested in RMS and pediatric glioma patients, with one ongoing Phase III glioma trial awaiting results. The Phase I/II INFORM2 trial of 2 mg/m² and 4 mg/m² (RP2D) entinostat plus nivolumab showed safety and tolerability of RP2D in 15 patients between 12 and 21 years old, with 1 observed DLT in 6 patients (grade 3 thrombocytopenia) (van Tilburg et al., 2022). The dose escalation portion of this study is ongoing for patients aged 6 to 11 years old. A Phase I trial of mocetinostat plus vinorelbine for recurrent or refractory RMS did not trigger any serious adverse events related to the treatment regimen. Of the 7 evaluable patients, 4 had PR; 2 had SD; and 1 had PD, with an overall clinical benefit rate of 86 percent. Rapid responses were seen in a majority of these patients at a median of 1.5 months. The duration of these responses was greater than 6 months for 4 of these patients, and the median duration of response (mDOR) was 8 months, with a range of 4 to 16 months (Federman et al., 2022). A Phase I trial of 30mM or 60mM panobinostat plus gadolinium administered using convection-enhanced delivery for newly-diagnosed DMGs in 7 patients triggered one serious adverse event due to infusion and tumor anatomy. PFS was between 8 and 20 months after last follow up between 12 and 22 months. One patient had an OR, and three patients survived (2 without progression at 1 year and 1 with PD at 22 months) (Zacharoulis et al., 2022).

**Research and Development Landscape**

NCI and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) administer the most HDAC1-3-related projects. However, less than half of HDAC1-3 literature addresses these proteins as potential oncology targets, and few research publications report relevant results for HDAC1-3 in pediatric cancers. Glioma, retinoblastoma, and AML are the most frequently studied cancer types in HDAC1-3 publications, while Wilms tumor, hepatoblastoma, and MRT are only referenced in one article each. Synovial sarcoma, ependymoma, pineoblastoma, MNST, and CCSK are not discussed in any HDAC1-3 research articles.

Drug development for HDAC1-3 is high relative to other oncology targets, with 4 approved drugs, 22 drugs in active development, and 23 planned and ongoing pediatric clinical trials.
Belinostat, romidepsin, and vorinostat are FDA-approved for treatment of T-cell lymphoma and panobinostat is FDA-approved for treatment of multiple myeloma. However, vorinostat clinical trials have yielded mixed data in other tumor types. A Phase II clinical trial in relapsed and recurrent neuroblastoma found that while combination therapies with vorinostat (e.g., MIBG plus vorinostat) showed greater treatment response than treatment without vorinostat (e.g., MIBG alone, MIBG plus irinotecan plus vincristine), PFS and overall survival were lower for patients receiving vorinostat compared other treatment arms, suggesting higher treatment response rates may not indicate overall utility for the addition of vorinostat to other treatment regimens (DuBois et al., 2021; New Approaches to Neuroblastoma Therapy Consortium, 2022). Another Phase II trial, in newly-diagnosed DIPG cases, found that combining vorinostat with radiation therapy was well tolerated but failed to improve treatment outcomes (Su et al., 2021), and in a Phase II/III trial of patients with HGGs treated with radiation plus vorinostat, temozolomide, or bevacizumab, those receiving vorinostat had a lower EFS rate compared to temozolomide (National Cancer Institute (NCI), 2023b).

Clinical trial accrual for pediatric studies using HDAC1-3 inhibitors is relatively low compared to accruals for other oncology targets. Therefore, these trials are likely more affordable and more feasible than pediatric trials for other drug targets.

**Additional Considerations for Preclinical and Clinical Development**

*Facilitated by Michelle Monje, MD, PhD, Stanford Medicine, and Charles Keller, MD, Children’s Cancer Therapy Development Institute*

Following Dr. Adam’s HDAC presentation, Drs. Michelle Monje and Charles Keller facilitated a discussion on PK study logistics, caveats for *in vitro* and *in vivo* preclinical models, single-agent activity, toxicity, and combination therapies. Meeting participants also discussed different mechanisms by which HDACs may exert therapeutic activities in specific pediatric indications.

**Funding Support for Pharmacokinetic Preclinical Studies**

PK results are critical for determining dosage and treatment schedule for clinical trials. Adult PK data are not always readily translatable to pediatric patients, and mouse PK studies are expensive, generate varied results based on strain, and do not readily recapitulate human metabolism. Therefore, researchers must consider which PK metrics are most useful in preclinical models. In addition, PK studies are expensive and not usually funded by NIH.

**Caveats to In Vitro and In Vivo Preclinical Models**

*In vitro* studies of HDAC inhibitors should use contemporary models (i.e., not cells that have been serially-passaged for decades) and multiple biological replicates. In addition, some cell lines are always sensitive to certain drugs; for example, panobinostat treatment results in false positives in some *in vitro* tumor cell lines. Ultimately, *in vitro* drug testing is difficult to directly translate to *in vivo* contexts due to different modes of drug administration. *In vivo* studies should also use multiple contemporary models that are representative of pediatric patients and useful for PK studies.
**Single-Agent Activity**

Every HDAC inhibitor is unique in terms of mechanism of action and side effect profile, even for two inhibitors targeting the same HDAC. In addition, HDAC inhibitors that are more specific to individual HDACs may be more effective than pan HDAC inhibitors. The increased selectivity of HDAC inhibitors may reduce toxicity and enable use of higher doses. The uniqueness of each inhibitor may require testing multiple inhibitors across multiple tumor types. However, instead of conducting a large-scale drug screen, basic research on the biology of different HDACs may provide insight into which HDAC drugs may be most effective in certain pediatric cancers.

Many HDAC inhibitors lack single-agent activity in some pediatric indications, but these same inhibitors may show strong therapeutic effect when administered as part of a combination therapy. One notable exception is that administration of HDAC inhibitors does result in peripheral T-cell lymphoma regression in adults. The attributes that make peripheral T-cell lymphoma sensitive to HDAC inhibition are not well understood, but HDAC inhibitors could exert single-agent therapeutic effects in pediatric tumors with similar sensitizing attributes.

Panobinostat demonstrated single-agent activity in pediatric fibrolamellar cancer and hepatoblastoma in xenografts. In addition, Dr. Monje explained that the single-agent activity of panobinostat was observed in a Phase II pediatric DIPG clinical trial. The dose-limiting toxicity for this study was hematological, but some enrolled patients temporarily and partially responded to panobinostat. Panobinostat’s effects were limited due to poor penetration of the BBB; thus, another HDAC inhibitor capable of efficiently crossing the BBB may be more successful in these DIPG patients than panobinostat. Unfortunately, another clinical trial of panobinostat directly injected into pediatric DIPGs had to stop early due to lack of panobinostat availability. The panobinostat manufacturer stopped manufacturing the drug, halting any further study despite single-agent activity.

**Toxicity**

HDAC inhibitors can have broad short- and long-term effects on gene expression, which could also trigger unintended effects on developmental processes and maintaining homeostasis in pediatric patients. For example, entinostat has been studied for over 20 years and used to treat breast cancer in Phase III clinical trials, but this drug causes nausea, fatigue, and diarrhea in 30 to 50 percent of patients. Notably, the side effect profile for children is quite different, likely due in part to differences between adult and pediatric metabolism; the half-life of entinostat in pediatric patients is much longer, and therefore, could only be administered weekly.

Novartis previously described how one of its HDAC inhibitors altered the expression of cardiac transporters, which led to cardiac abnormalities. Dr. Smith expressed concern that these cardiac effects may be a shared effect across multiple HDAC inhibitors. However, the route of administration greatly impacts cardiotoxicity. For example, QTc prolongation and cardiotoxicity with panobinostat was primarily observed when the drug was administered intravenously, while oral administration resulted in less frequent cases of QTc prolongation. Ultimately, proteolysis targeting chimeras (PROTACs) and molecular glues will eventually enable HDAC to target specific genetic loci, potentially minimizing toxic effects.
**Matching Unique HDAC Inhibitors to Different Tumor Types**

Epigenetic dysregulation is a more prevalent cancer driver in pediatric patients compared to adults. However, dysregulation vastly differs across tumor types. Thus, unique HDAC inhibitors with diverse mechanisms of action should be matched to specific tumor pathobiologies.

**Rhabdomyosarcoma**

In RMS, PAX3/FOXO expression is necessary but not sufficient for tumor initiation, dispensable for tumor maintenance, and critical for developing chemotherapy resistance via cell cycle checkpoint regulation. Increased PAX3/FOXO promotes transition through the G2/M cell cycle transition, enabling tumor cells to evade apoptotic signals and proceed through cell division. HDAC inhibitors could repress inappropriate expression of PAX3/FOXO to address chemotherapy resistance.

**Diffuse Intrinsic Pontine Glioma**

In DIPGs, HDAC activity has a role in tumor maintenance; HDAC inhibitors have resulted in regression in some preclinical models as well as patients. HDAC inhibition synergizes with proteasome inhibitors to induce metabolic collapse that is rescuable with nicotinamide adenine dinucleotide (NAD⁺) repletion. HDAC inhibitors may also restore gene expression by reversing the loss of K27 trimethylation on histone 3 (H3). In addition, mutations to H3 can cause dysregulation of polycomb repressive complex 2 (PRC2), which impacts epigenetic regulation. However, these DIPG-specific mechanisms are still not well understood.

**Neuroblastoma**

HDAC inhibitors followed by stem cell rescue—when combined with meta-iodobenzylguanidine (MIBG)—are becoming standard of care for some neuroblastoma patients. Two mechanisms could explain this combination’s success: (1) HDAC inhibition enhances the effect of MIBG’s DNA damage effects, or (2) HDAC inhibition increases MIBG receptor expression, resulting in tumor sensitization to MIBG. However, vorinostat plus MIBG resulted in a shorter—but not statistically significant—PFS compared to patients that received MIBG alone, although the response rate was higher for the combination arm. Larger studies with more specific patient populations are needed to understand whether adding vorinostat to MIBG provides added clinical benefit. Notably, the genetic background of these tumors may affect HDAC inhibition sensitivity; loss of a single copy HDAC1 sensitize tumors to HDAC2 inhibition, and vice versa.

**Combination Therapies**

Meeting participants discussed the following potential combination therapeutic strategies for HDAC inhibitors:

- **Conventional chemotherapy**: Use of HDAC inhibitors may reduce chemotherapy doses necessary for therapeutic effects. Preclinical work should be conducted in mice to assess whether lower chemotherapy doses are effective with specific HDAC inhibitors.
- **Proteasome inhibitors**: Further preclinical research is needed to understand mechanisms by which HDAC and proteasome inhibitors may exert synergistic therapeutic effects.
- **MEK inhibitors**: Synergism of MEK inhibitors with HDAC inhibitors has been observed *in vitro*, but further *in vivo* research is needed to understand whether thrombocytopenia limits MTD below a threshold for therapeutic effect.
- **Targeted immunotherapies**: Specific HDAC inhibitors may alter expression of immunotherapy targets, resulting in sensitization of tumors to these immunotherapies.

**Next Steps**
HDAC1, 2, and 3 are high priority drug targets. Moving forward, robust *in vitro* screens informed by biological mechanisms of specific tumor types and inhibitor mechanisms of action can help identify HDAC inhibitors that may be effective in pediatric indications. Further *in vivo* research using multiple biological replicates can confirm whether an HDAC inhibitor is effective for a particular indication. The scientific community needs additional investments in PK and animal testing to bridge preclinical research findings with clinical drug development; one strategy for bridging this preclinical-clinical gap involves collaboration between academic and industry laboratories with PIVOT. In addition, identification of CNS-penetrant HDAC inhibitor strategies will increase efficacy in CNS tumors. Lastly, computational approaches analyzing PRISM data and *in vivo* HDAC inhibitor data can help identify biomarkers that may explain why certain PDXs are sensitive to HDAC inhibition.
Appendix A: Feedback

Ms. Tetyana Murza facilitated a feedback session with meeting participants to discuss pre-meeting materials, COACH meetings, and the target selection process. Multiple meeting participants appreciated pre-meeting materials as thorough summaries of each field of study. In addition, expert-led discussions have brought additional nuance needed to assess drug targets. Ms. Murza also accepts feedback via email.

The next COACH meeting will occur on June 13, 2023, from 10am to 2pm ET. Participants will discuss BCL-2 proteins—BCL-2, myeloid cell leukemia 1 (MCL-1), and B-cell lymphoma-extra large (BCL-XL). Ms. Murza and Dr. Adam request volunteers and recommendations for experts to lead data presentations and facilitate subsequent discussions.

The next round of target nomination and voting—for the September 2023 meeting—will begin in early April. Organization leads may provide two to three target nominations in response to an email from FNIH requesting nominations. Alternatively, meeting participants can review ADCs during the September meeting (ranked second behind BCL-2 in the previous vote). Currently, there is limited activity in the ADC space for CD19, CD22, CD33, and CD123. Meeting participants should consider which of these proteins to focus on during a potential ADC meeting. FNIH will send meeting participants an email to request preferences for specific ADCs.
Appendix B: Bibliography


Convening Experts in Oncology to Address Children’s Health

March 15, 2023


Jonsson Comprehensive Cancer Center. (2022). *A Phase I Dose Escalation/Expansion Clinical Trial of Mocetinostat in Combination With Vinorelbine in Children, Adolescents and Young Adults With Refractory and/or Recurrent Rhabdomyosarcoma (RMS)* (Clinical Trial Registration No. NCT04299113). clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT04299113


Ma, P., Pan, H., Montgomery, R. L., Olson, E. N., & Schultz, R. M. (2012). Compensatory functions of histone deacetylase 1 (HDAC1) and HDAC2 regulate transcription and apoptosis during mouse oocyte development. *Proceedings of the National Academy of
Convening Experts in Oncology to Address Children’s Health
March 15, 2023

*Sciences of the United States of America, 109*(8), E481-489. https://doi.org/10.1073/pnas.1118403109


Appendix B
National Cancer Institute. (n.d.). *Sirolimus to Treat Cowden Syndrome and Other PTEN Hamartomatous Tumor Syndromes.* https://classic.clinicaltrials.gov/ct2/show/NCT00971789


Appendix B


