Convening Experts in Oncology to Address Children’s Health

Quarterly Collaboration Meetings in Pediatric Oncology

July 22, 2022 | Virtual Meeting

Reviewed Targets:
EZH2
MDM2
CD47

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July 22, 2022

Acronym Definitions

COACH Convening Experts in Oncology to Address Children's Health
ALK anaplastic lymphoma kinase
ALL acute lymphoblastic leukemia
ALT Alternative Lengthening of Telomeres
AML acute myeloid leukemia
ATRT atypical teratoid rhabdoid tumor
ATRX alpha thalassemia X-linked
AUC area under the curve
B-ALL B-cell acute lymphoblastic leukemia
BBB blood-brain barrier
CD47 Cluster of differentiation 47
CNS central nervous system
CNV copy number variation
DCR disease control rate
DepMap Dependency Map
DZNep 3-Deazaneplanocin A
EMA European Medicines Agency
EZH2 enhancer of zeste homolog 2
FDA Food and Drug Administration
ITCC-P4 Paediatric Preclinical Proof of Concept Platform
JMML juvenile myelomonocytic leukemia
mAb monoclonal antibody
MDM2 murine double minute 2
MDMX/MDM4 murine double minute X
MDS myelodysplastic syndrome
MHC major histocompatibility complex
MRT malignant rhabdoid tumor
NCI National Cancer Institute
NSD2 Su(var)3-9 enhancer-of-zeste and trithorax domain protein 2
ORR objective response rate
PDX patient-derived xenograft
PIVOT Pediatric Preclinical In Vivo Testing
PRC2 Polycomb Repressive Complex 2
PRISM Profiling Relative Inhibition Simultaneously in Mixtures
SEER Surveillance, Epidemiology, and End Results
SIRPα signal regulatory protein alpha
SMARCA4 switch/sucrose non-fermentable related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4
SMARCB1 switch/sucrose non-fermentable related, matrix associated, actin dependent regulator of chromatin, subfamily B, member 1
SSM simple somatic mutation
SV structural variation
T-ALL T-cell acute lymphoblastic leukemia
TP53 tumor protein 53
TROP2 tumor-associated calcium signal transducer 2
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Executive Summary

Despite advances in therapeutic development for adult cancers, development of treatment regimens for pediatric cancers poses unique challenges, in part, because effective adult treatments are not always readily translatable to the pediatric population due to distinct differences between adults and children even with the same cancer diagnosis. In addition, patient populations for specific pediatric cancers are often quite small, which complicates study design and sufficient powering for pediatric clinical trials. Convening Experts in Oncology to Address Children’s Health (COACH) will assemble subject matter experts from diverse fields to review research landscapes for therapeutic targets of potential interest for pediatric oncology drug development and will offer recommendations regarding preclinical research activities to further develop therapeutics for use in pediatric populations. On July 22, 2022, COACH convened the First Quarterly Collaboration Meeting representatives from National Cancer Institute (NCI), Food and Drug Administration (FDA), European Medicines Agency (EMA), advocacy groups, 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 for development of three drug targets for early phase pediatric clinical trials: enhancer of zeste homolog 2 (EZH2), mouse double minute 2 (MDM2), and cluster of differentiation 47 (CD47).

EZH2

Based on comparison of common alterations between adult and pediatric cancers, switch/sucrose non-fermentable related, matrix associated, actin dependent regulator of chromatin, subfamily B, member 1 (SMARCB1) and subfamily A, member 4 (SMARCA4) expression and genomic alterations may be more relevant than EZH2 mutation status for EZH2 pathway-related pediatric cancers. Meeting participants discussed several indications for which EZH2 inhibitors could be a promising therapeutic for pediatric cancers, including atypical teratoid rhabdoid tumor (ATRT), malignant rhabdoid tumor (MRT), and chordoma. With additional preclinical research, EZH2 inhibitors could prove effective for other pediatric indications including acute lymphoblastic leukemia (ALL), glioma, medulloblastoma, rhabdomyosarcoma, and some central nervous system (CNS) tumors.

Key Considerations

● Novel uses for EZH2 inhibitors include pre-treatment of relapsed ALL patients with a mutation in binding Su(var)3-9 enhancer-of-zeste and trithorax domain protein 2 (NSD2) to reverse glucocorticoid resistance and also enhancement of immunotherapies that require major histocompatibility (MHC) class 1 antigen expression.
● Successful treatment of pediatric indications with EZH2 inhibitors may require combination strategies with chemotherapy, immunotherapy, tyrosine kinase inhibition, or monoclonal antibody (mAb) therapy. Further preclinical research will provide insight into effective combinations and dosing regimens.
● In addition to EZH2 inhibitors, other therapeutics, including embryonic ectoderm development inhibitors, can also be used to inhibit EZH2 activity.
**Next Steps**

- Expand preclinical testing to other pediatric indications, including neuroblastoma, alpha thalassemia/mental retardation syndrome X-linked (ATRX)-driven tumors, and MYC-N-driven tumors.
- Identify biomarkers that predict EZH2 inhibition sensitivity.
- Use immunocompetent preclinical models to study EZH2 inhibitor combinations, especially immunotherapies.
- Perform preclinical research to understand the effects of EZH2 inhibition on immunogenic pathways and the long-term effects of its inhibition on the immune system.

**MDM2**

MDM2 inhibitors should only be used to treat tumors that are wildtype for the downstream tumor protein 53 (TP53). Meeting participants discussed several indications for which MDM2 inhibitors could be a promising therapeutic for pediatric cancers, including rhabdoid tumors, some soft tissue sarcomas, ALL, and some CNS tumors. Additional preclinical research exploring the efficacy and applicability of MDM2 inhibitors for treatment of MRT, blood cancers including ALL and AML, malignant peripheral nerve sheath tumor, and synovial sarcoma may contribute to MDM2 inhibitor development for pediatric indications.

**Key Considerations**

- Toxicity concerns limit systemic administration to MDM2 inhibitors. Further understanding of MDM2 inhibitor effects on blood cell populations, particularly platelet progenitors, may provide insights to mitigate toxicity.
- One therapeutic objective for MDM2 inhibitors is to sufficiently sensitize tumors to chemotherapy and other treatments, though achieving this objective may be difficult due to dose-limiting hematologic toxicity before adequate blood concentrations are achieved.
- When developing MDM2 inhibitor treatment regimens, researchers and clinicians need to consider acquisition of MDM2 inhibitor resistance.
- Identifying predictive biomarkers for sensitivity to MDM2 inhibition could help clinicians identify patients most likely to respond to MDM2 inhibitor treatment.

**Next Steps**

- Test MDM2 inhibitor treatment in combination with chemotherapy, such as venetoclax, and radiation.
- Evaluate efficacy of second-generation MDM2 inhibitors in preclinical models of indications that are expected to respond to MDM2 inhibition (e.g., wildtype TP53 tumors, neuroblastoma, hematologic cancers).

**CD47**

Meeting participants discussed several indications for which anti-CD47 therapeutics could be promising for the treatment of ALL, glioma, and osteosarcoma. Additional preclinical research may identify other anti-CD47-sensitive pediatric cancers.
Key Considerations
● Anti-CD47 therapeutics may be more effective in combination with chemotherapy, immunotherapy, and mAb therapy, with particular focus being given to the ability of anti-CD47 therapeutics to enhance the antibody-dependent cellular cytotoxicity effects of therapeutic monoclonal antibodies.
● When developing pediatric cancer treatment regimens, researchers and clinicians should consider relevant clinical research and development of anti-CD47 therapies in adult acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and Hodgkin’s and non-Hodgkin’s lymphoma.
● Signal regulatory protein alpha (SIRPα) expression levels in macrophages may alter the effectiveness of anti-CD47 therapeutics.

Next Steps
● Assess preclinical efficacy of CD47 inhibition in models of sarcoma and ALL.
● Characterize SIRPα expression patterns and determine whether these expression patterns provide insight into CD47 inhibitor sensitivity.
● Conduct preclinical research to identify CD47 inhibitor combination treatments, including combinations with standard chemotherapies.
Children's Cancer Incidence and Mortality: Relevance to Pediatric Drug Development

Malcolm Smith, PhD, National Cancer Institute

To determine the safety and efficacy of therapeutics, clinical trials require sufficient patient enrollment. Moreover, participation in early phase pediatric oncology clinical trials typically requires that patients have exhausted all standard treatment options and have experienced cancer recurrence or refractory disease, reducing the population size. However, no United States entity tracks the rates of pediatric cancer recurrence and refractory disease by cancer type. Thus, it is challenging to determine whether the eligible patient population for a given indication will be sufficient to meet necessary recruitment targets for clinical trials.

Because disease relapses highly correlate with eventual death for many pediatric cancers, mortality calculations can serve as a surrogate for patients experiencing relapse or refractory disease to estimate whether a patient population is large enough to sufficiently power an early phase clinical trial. To estimate the number of pediatric oncology patients potentially eligible for early phase clinical trials, Dr. Smith presented a method to estimate pediatric cancer mortality as a proxy for pediatric patients with recurrence or refractory disease.

Method for Estimating Pediatric Cancer Mortality

Surveys of death certificates alone in the United States cannot provide complete pediatric cancer mortality data because death certificates do not document childhood cancer diagnoses with sufficient granularity. However, cancer registries such as the National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) Program and the National Childhood Cancer Registry (NCCR) compile annual pediatric cancer incidence data as well as survival data, and these can be used to estimate annual mortality by specific cancer types.¹

The National Childhood Cancer Registry (NCCR) is a public health surveillance data resource that is being developed through the NCI Childhood Cancer Data Initiative (CCDI). The NCCR uses the Virtual Pooled Registry Cancer Linkage System to link multiple cancer registries and generate an accurate count of childhood cancer cases by combining information that appears in more than one registry. The NCCR is currently comprised of 24 state and regional cancer registries, and it represents two thirds of all U.S. children under the age of 20.

¹ Mortality estimations used the following equations:

\[
\text{annual incidence} = \text{annual diagnoses per 1,000,000 children} \times \text{total child population}
\]

\[
\text{five-year mortality rate} = 1 - (5\text{-year survival rate})
\]

\[
\text{annual mortality} = \text{annual incidence} \times 5\text{-year mortality rate}
\]
Pediatric Cancer Mortality for Select Cancer Types

Dr. Smith presented incidence data and mortality estimates using NCCR data for neuroblastoma, juvenile myelomonocytic leukemia (JMML), atypical teratoid/rhabdoid tumor (ATRT), non-central nervous system (CNS) rhabdoid tumors, T-cell acute lymphoblastic leukemia (T-ALL), and B-cell acute lymphoblastic leukemia (B-ALL), summarized in the table below. The estimated number of cases for each cancer type are based on the cancer incidence and on the 2020 USA population for the relevant age cohort. ²³

<table>
<thead>
<tr>
<th></th>
<th>Neuroblastoma (0-14 years)</th>
<th>JMML (0-14 years)</th>
<th>ATRT (0-14 years)</th>
<th>Non-CNS rhabdoid (0-14 years)</th>
<th>T-ALL (0-19 years)</th>
<th>B-ALL (0-19 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Annual incidence (per 1,000,000)</strong></td>
<td>11.065</td>
<td>0.375</td>
<td>1.232</td>
<td>0.483</td>
<td>4.196 (0-14 years)</td>
<td>36.799 (0-14 years)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.083 (15-19 years)</td>
<td>15.244 (15-19 years)</td>
</tr>
<tr>
<td><strong>Estimated total annual diagnoses</strong>³</td>
<td>667</td>
<td>23</td>
<td>74</td>
<td>29</td>
<td>253 (0-14 years)</td>
<td>2,219 (0-14 years)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65 (15-19 years)</td>
<td>320 (15-19 years)</td>
</tr>
<tr>
<td><strong>5-year mortality rate</strong></td>
<td>16.7%</td>
<td>35.2%</td>
<td>55.6%</td>
<td>63.6%</td>
<td>13.5% (0-14 years)</td>
<td>7.7% (0-14 years)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19.5% (15-19 years)</td>
<td>23% (15-19 years)</td>
</tr>
<tr>
<td><strong>Estimated total annual deaths</strong></td>
<td>111</td>
<td>8</td>
<td>41</td>
<td>18</td>
<td>47</td>
<td>244</td>
</tr>
</tbody>
</table>

Considerations for Pediatric Drug Development

Using mortality as a proxy for cancer recurrence and refractory disease likely underestimates the number of patients available for clinical trials. The extent of underestimation varies across tumor types. For example, underestimation is greatest for cancers with both high rates of relapse and high long-term survival; those patients could be eligible for early-phase clinical trials but are not captured in these estimates. By contrast, there are also factors that may reduce the number of patients that proxy mortality estimates indicate are available for early phase clinical trials, such as: (1) ineligibility due to sickness from prior treatments; (2) patient family reluctance; (3) rarity of molecular cancer subtypes; and (4) reduced number of open study sites. Opening enrollment to Europe could double the number of eligible early phase trial participants. Mortality rates alone do not reflect the entirety of drug development needs; many

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² Calculations assume a population in 2020 of 60,293,426 children ages 0-14 years and 20,960,929 for children ages 15-19 years.
³ Calculations assume a population in 2020 of 60,293,426 children ages 0-14 years and 20,960,929 for children ages 15-19 years.
⁴ Calculations assume a population in 2020 of 60,293,426 children ages 0-14 years and 20,960,929 for children ages 15-19 years.
long-term pediatric cancer survivors experience long-term complications and toxicities, and they may benefit from the availability of effective treatments that have fewer complications.

Dr. Smith requested feedback from meeting participants related to incidence and mortality data: (1) increasing utility to drug developers; (2) increasing public availability; and (3) presenting insightful visualizations.

**Discussion**

Dr. Mike Dyer noted that SEER*Explorer, a portal that accesses various cancer statistics, does not separate incidence and mortality rate data by cancer type. Dr. Smith explained that software like SEER*Stat queries the SEER database by specific diagnostic codes. Incidence and mortality data by cancer type, if useful, could be made accessible to researchers.

Dr. John Maris explained that Dr. Smith’s estimates do not reflect relapsed/refractory high-risk disease and asked whether Dr. Smith and his group could estimate this as well. However, Dr. Smith noted this estimate may not be possible with the currently available NCCR*Explorer data.

**Pediatric Cancer Drug Target Data**

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

Dr. Adam presented data on patient alterations, patient expression, and in vitro and in vivo dependency for target oncogenes (i.e., enhancer of zeste homolog 2 [EZH2], mouse double minute 2 [MDM2], and cluster of differentiation 47 [CD47]), as well as data on in vitro and in vivo drug sensitivity and clinical response rates for their respective inhibitors. 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 inhibitors. 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 target a differentially expressed protein in a specific tumor type may not result in any significant therapeutic effect. While there are limitations to in vitro dependence data, they can provide scientific rationale to support further preclinical assessments. Moreover, in vitro dependency may not always reflect in vitro drug sensitivity.

Data types for each drug target were compiled from different databases as well as scientific literature. Compiled patient alteration data included simple somatic mutations (SSMs), copy number variations (CNVs), and structural variations (SVs) compiled from cBioPortal and PedcBioPortal cohorts. Patient expression data was compiled from CCDI Molecular Targets Platform and XenaBrowser. In vitro dependency data were presented using Chronos scores, a normalized metric of cell viability after gene deletion, obtained from Dependency Map (DepMap). 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 was 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.

**EZH2**

*Stacey Adam, PhD, FNIH*

**Overview**

EZH2 is a component of the Polycomb Repressive Complex 2 (PRC2), which epigenetically regulates various genes involved in development and differentiation. Various tumor types often overexpress EZH2, which can result in the inappropriate suppression of regulators that would normally inhibit tumor growth.

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

The activating mutations in EZH2 that occur in adults with NHL are rarely observed in pediatric cancers. While the COSMIC database contains 782 EZH2 mutations detected adult tumor samples, only 70 EZH2 mutations were detected in over 5,000 pediatric tumor samples from St. Jude’s Pediatric Cancer Data Portal. Only 17 of the 70 pediatric mutations were also detected in adult tumors. Therefore, EZH2 inhibitor data from adult cancers for patients with activating EZH2 mutations has limited applicability to pediatric cancers.

Alterations in the target may not always be the most relevant alterations. Because switch/sucrose non-fermentable related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4 (SMARCA4) and subfamily B, member 1 (SMARCB1) mutations are often detected in tumors with EZH2 dependency, patient alteration analysis also included these genes. A high number of SMARCA4 and SMARCB1 alterations are present in MRTs (Chun et al., 2016; Lee et al., 2012), and a subset of osteosarcomas contains SMARCA4 alterations. In addition, pediatric ATRTs, MRTs, and chordomas often contain mutations resulting in loss of SMARCA4 or SMARCB1, and SMARCA4 alterations have been detected in a small subset of neuroblastomas. Loss of SMARCA4 and SMARCB1 may be more relevant than EZH2 activation for pediatric cancers.

EZH2 mRNA expression is highly variable across pediatric primary tumor types, with heightened expression in clear cell sarcoma of the kidney, neuroblastoma, and Wilms’ tumor, and reduced expression in a small number of MRTs. Consistent with the high rate of SMARCB1 mutation, SMARCB1 expression is lowest in ATRTs, MRTs, and chordomas compared to other pediatric cancer types. MRTs often express lower levels of SMARC4A as well.

**In Vitro and In Vivo Dependency**

EZH2 deletion significantly reduces cell viability in MRT, acute lymphoblastic leukemia (ALL), medulloblastoma, and neuroblastoma cell lines, but does not impact viability in Ewing sarcoma, glioma, and osteosarcoma cell lines. However, the in vivo dependency for EZH2 is poorly understood. While loss of EZH2 drives ALL progression, loss of EZH2 blocks tumor formation in the SMARCB1 T-cell lymphoma model (Wilson et al., 2010).
In Vitro and In Vivo Drug Sensitivity

In vitro drug sensitivity data for EZH2 inhibitors do not replicate in vitro dependency data. Despite evidence for in vitro dependency for EZH2 in MRTs, 3-Deazaneplanocin A (DZNep) exposure does not reduce MRT cell line viability. Moreover, DZNep, a small molecule EZH2 inhibitor, only slightly reduces viability across other cancer cell lines. This slight reduction of cell viability is recapitulated with another EZH2 inhibitor, tazemetostat. Notably, the PRISM drug sensitivity assay protocol involves incubation in drugs of interest for only five days, while other EZH2 inhibitor studies have reported significant reduction in cell viability after 7-14 days of incubation (Knutson et al., 2014; Knutson et al., 2013), suggesting that longer treatment times may be needed. Dr. Yael Mosse explained that her research group, in collaboration with Pfizer, detected sensitivity to a novel EZH2 inhibitor in some in vitro models, including alpha thalassemia X-linked (ATRX)-driven models of neuroblastoma (following on work from the Bernstein laboratory), neuroblastoma models with the Alternative Lengthening of Telomeres (ALT) phenotype without ATRX alteration, and triple negative breast cancer cells with ALT phenotype (Qadeer et al., 2019). In vivo testing in neuroblastoma PDX models will be initiated soon.

In vivo models for glioma, medulloblastoma, rhabdomyosarcoma, and MRT exhibit drug sensitivity to EZH2 inhibitors. In addition, a patient-derived xenograft (PDX) mouse model of synovial sarcoma with synovial sarcoma translocation that disrupts SMARCB1 responded to tazemetostat (Kawano et al., 2016).

Clinical Response Rates

EZH2 inhibitor responses in some adult clinical trials were consistent with in vivo data suggesting in vivo drug sensitivity for certain cancer types. Of six pediatric patients with ATRTs and other rhabdoid tumors treated with EZH2 inhibitors, two tumors showed delayed growth and one patient achieved stable disease. In a Phase I adult study, treatment of relapsed or refractory SMARCB1 tumors with tazemetostat resulted in detectable disease control rates for ATRT (33% DCR, 24% objective response rate [ORR]), non-ATRT (14% DCR, 0% ORR), Ewing sarcoma (67% DCR, 22% ORR), and chordoma (50% DCR, 33% ORR) (Epizyme, 2021). Recent results of a Phase II trial of tazemetostat demonstrated a 38 percent DCR and 5 percent ORR in tumors containing EZH2 hotspot tumors or loss of SMARCB1 or SMARCA4 (National Cancer Institute, 2022a). Due to the highly variable response in pediatric tumors of the same type to EZH2 inhibition, Drs. Susan Weiner and Mark Kieran suggested that conducting additional preclinical studies may identify genotypes and phenotypes that predict response to EZH2 inhibition.

Dr. Kieran noted that while in vivo animal models showed no objective response to EZH2 inhibition, certain relapsed, poor prognosis patients have completely or partially responded to EZH2 inhibition, suggesting that current in vivo models may not represent and translate the

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5 disease control rate=partial response rate+complete response rate+maintained complete response rate+stable disease rate

6 objective response rate=partial response rate+complete response rate+maintained complete response rate+stable disease rate
results to humans. Dr. Smith explained that in preclinical subcutaneous MRT models, treatment with tazemetostat did shrink tumors, but only after initial tumor growth during the first two weeks of treatment. However, tumors have a more rapid growth, and the subsequent tumor shrinkage did not meet the criteria for an objective response. Dr. Dyer further explained that discrepancies between patient responses to EZH2 inhibition and data generated using in vivo models may result from unresolved pharmacokinetics and dosing schedule differences across mouse and human protocols.

Novel use cases for EZH2 inhibitors include (1) treatment of a subset of pediatric ALL patients and (2) enhancement of immuno-oncology agents that require expression of major histocompatibility complex (MHC) class 1 antigens. One preclinical study found that EZH2 inhibitors reversed glucocorticoid resistance in some ALL cancer models (Li et al., 2022). Because nuclear receptor binding Su(var)3-9 enhancer-of-zeste and trithorax domain protein 2 (NSD2) mutations can drive glucocorticoid resistance in relapsed pediatric ALL patients, pretreating with EZH2 inhibitors may improve second line therapeutic strategies. However, Dr. Smith noted that only a small population of pediatric ALL patients contain relevant NSD2 mutations, complicating the sufficient powering of clinical studies. In addition, the pediatric ALL field is crowded, and the effect of EZH2 inhibition may not be sufficient to warrant further development. By contrast, Dr. Gregory Reaman suggested that clinicians could identify all ALL patients with NSD2 mutations either at initial diagnoses or time of relapse, enabling enrollment in an EZH2 inhibitor clinical trial. Another preclinical study demonstrated that EZH2 inhibitors increase MHC class 1 antigen presentation on tumor cells (Zhou et al., 2020). Therefore, EZH2 may enhance the effect of some immunotherapies that require MHC class 1 antigen-expressing tumor cells.

**Additional Considerations for Preclinical and Clinical Development**

After the conclusion of Dr. Adam’s EZH2 presentation, meeting attendees further discussed potential EZH2 treatment combinations and additional EZH2 complex inhibitors.

It was also commented that the high relevance of understanding why some patients respond, and others do not, with the same mutation (e.g., by genotyping responding vs non-responding tumors) may guide additional targeted therapies.

**Treatment Combinations**

Although few EZH2 combination treatment studies have been conducted to date, meeting attendees agreed that EZH2 inhibitors will perform best in combination with other existing cancer therapies. For example, recent results from an EZH2 clinical trial conducted by Susan Chi (Harvard Medical School) showed dramatic response to EZH2 inhibition followed by quick recurrence in patients with ATRT, chordoma, and epithelioid sarcoma, suggesting successful treatment with EZH2 inhibitors may require additional therapy to prevent recurrence. Some attendees agreed that more preclinical research is needed regarding combinations, to identify what are the “rational” combination partners and to validate the mechanistic hypothesis from these combinations.
Chemotherapy—Dr. Maris observed that companies are reluctant to engage in pediatric cancer studies using drugs with a risk of secondary malignancy (e.g., leukemia). Therefore, Dr. Andy Pearson suggested that such treatment plans consider including combination therapy to mitigate secondary malignancy risks. Moreover, by further understanding EZH2 inhibitor mechanisms of action, secondary malignancy risk can be better understood and reduced with certain treatment schedules. Preclinical studies can help researchers establish dose schedules and chemotherapy combinations for rhabdoid tumors while anticipating potential toxicities in the clinic. However, Dr. Beth Stewart noted that enrollment in early phase clinical trials would require extensive collaboration to sufficiently power a treatment combination study due to the small eligible patient population for rhabdoid tumors. Dr. Stergios Zacharoulis suggested the group prioritize combination treatments between EZH2 and more targeted therapies, such as methylating agents, histone deacetylase inhibitors, and checkpoint inhibitors, rather than EZH2 and standard chemotherapy.

Immunotherapy—Drs. Maris and Mosse expressed interest in combining EZH2 inhibitor therapy with other immunotherapies to harness T-cell mediated anti-tumor immunity. However, Dr. Smith noted immune checkpoint inhibitors have been largely unsuccessful in pediatric cancers. Dr. Maris is developing synthetic immunotherapies that require MHC class 1 antigen-expressing tumor cells and that may work better than immune checkpoint inhibitors in conjunction with EZH2 inhibitors. Finally, the only FDA approved immunotherapies for pediatric solid cancer are GD2-directed monoclonal antibodies. Loss of GD2 expression may be a mechanism of resistance to this class of antibodies. Dr. Nathalie Scholler noted that a recent publication showed synergy between anti-GD2 and anti-CD47 antibodies, which subsequently can activate the innate immunity (Theruvath et al., 2022). Dr. Maris further explained that a recent collaboration between the Stegmaier and Majzner laboratories showed that EZH2 inhibition can reverse GD2 loss due to epigenetic reprogramming of cellular state and suggested this combination for clinical testing (Mabe et al., 2022).

Tyrosine Kinase Inhibition—Dr. Mosse’s research group has shown EZH2 inhibition can modulate surface expression of anaplastic lymphoma kinase (ALK) on neuroblastoma cells, which can then be targeted with specific tyrosine kinase inhibitors. Her research group is currently treating N-Myc amplified tumors with an EZH2 inhibitor and a best-in-class ALK inhibitor for neuroblastoma (i.e., lorlatinib).

Additional EZH2 Complex Inhibitors
Dr. Kieran suggested that the group consider other strategies for inhibiting the EZH2 complex, including embryonic ectoderm development inhibitors that are currently in clinical trials. He also suggested investigation of dual inhibition of EZH1 and EZH2 for cancers with loss of SMARC1B or SMARC4A.

Next Steps
Meeting participants identified EZH2 as a high priority candidate drug target for future pediatric development. Currently, pediatric preclinical and clinical development is focused on SMARCB1-deficient tumors and soft tissue sarcomas, but EZH2 inhibition may also be effective in other...
pediatric indications. Preclinical research in other indications, such as neuroblastoma, ATRX-driven tumors, and MYC-N-driven tumors, could identify additional pediatric indications that may respond to EZH2 inhibition. Additional preclinical research could also help identify biomarkers that predict response to EZH2 inhibition.

EZH2 inhibition likely will not be sufficient as a monotherapy; thus, preclinical research is needed to identify EZH2 treatment combinations, especially with immunotherapies. Notably, preclinical testing of combinations with immunotherapies will require relevant immunocompetent mouse models. Available pharmacokinetic information on existing immunotherapies and EZH2 inhibitors can be used to identify potentially compatible combinations.

EZH2 inhibition may be a powerful way to upregulate immunogenic pathways and key cell surface molecules (e.g., GD2, MHC, ALK, tumor-associated calcium signal transducer 2 [TROP2]) that can then be targeted with other therapeutics. Further research is needed to understand the direct effects of EZH2 on immune cells, as well as any long-term effects of EZH2 inhibition on the immune system.

**MDM2**

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

**Overview**

MDM2 is an E3 ubiquitin-protein ligase that negatively regulates p53 transcriptional activity through ubiquitination leading to p53 degradation. Genetic amplification and mutations in the promoter of MDM2 result in the upregulation of its expression in many different tumor types.

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

Some adult and pediatric gliomas (5.7%) and osteosarcomas (10.2%) contain MDM2 alterations. Most MDM2 alterations in gliomas are amplifications, and all MDM2 alterations in osteosarcoma are amplifications. However, because MDM2 directly inhibits protein 53 (p53, encoded by tumor protein 53 [TP53] gene), MDM2 amplifications are only relevant in the presence of functional p53. Most pediatric cancers occur on wildtype TP53 backgrounds, but notable exceptions of cancers containing TP53 mutations include low hypodiploid ALL (91%), adrenocortical carcinoma (73%), diffuse intrinsic pontine glioma (65%), and non DIPG high-grade glioma (40%), as well as most osteosarcomas (Chen et al., 2014). MDM2 inhibitors would have no effect in these cancers lacking functional p53; therefore, Dr. Hubert Caron requested analysis of MDM2 in the context of TP53 status.

MDM2 is broadly increased expression across pediatric cancer types, especially in neurological cancers and Wilms’ tumor.

**In Vitro and In Vivo Dependency**

Dr. Adam presented in vitro dependency data for MDM2 only from wildtype TP53 cell lines. In vitro dependency data strongly support MDM2 dependency in soft tissue sarcoma, central CNS
tumors, MRTs, and blood cancers. Other in vitro studies implicate MDM2 in malignant peripheral nerve sheath tumor, synovial sarcoma, and retinoblastoma; further research may show stronger, more consistent evidence for in vitro MDM2 dependency in these cancer types.

Researchers have performed very few in vivo dependency studies for MDM2 across wildtype TP53 pediatric cancer indications. Some preliminary in vivo evidence recapitulates in vitro MDM2 dependency data for glioma and retinoblastoma. Further preclinical MDM2 in vivo dependency studies may help discern which pediatric cancer indications with demonstrated in vitro dependency on MDM2 may respond to MDM2 inhibitors.

**In Vitro and In Vivo Drug Sensitivity**

In vitro drug sensitivity data for MDM2 inhibitors from wildtype TP53 cell lines do not support in vitro MDM2 dependency data. Treatment of cell lines with an MDM2 inhibitor (i.e., RITA) resulted in loss of viability for five Ewing sarcoma lines and one hepatoblastoma line, while a second MDM2 inhibitor (i.e., serdemetan) reduced viability for Ewing sarcoma and osteosarcoma cell lines. Notably, MDM2 inhibitor sensitivity data were lacking for retinoblastoma and synovial sarcoma cells, despite the implication for MDM2 involvement based on in vitro dependency data.

Wildtype TP53 in vivo models of ALL, glioma, medulloblastoma, neuroblastoma, and osteosarcoma have been described as responding to treatment with MDM2 inhibitors. However, osteosarcoma sensitivity in many publications is due to the use of a uniquely sensitive MDM2-sensitive osteosarcoma cell line. In testing by the Pediatric Preclinical Testing Consortium (PPTC), ALL PDX mouse models were sensitive to two of MDM2 inhibitors—MK-8242 and RG7112 (Carol et al., 2013; Kang et al., 2016; Richmond et al., 2015). Some PDX models for rhabdoid tumors or Wilms’ tumors partially responded to these MDM2 inhibitors. Unsurprisingly, pediatric osteosarcoma patients with inactivated TP53 did not respond to MDM2 inhibitors.

**Patient Complications**

Scientific literature documents systemic negative side-effects from systemic administration of MDM2 inhibitors, including thrombocytopenia and thrombosis due to deleterious effects on hematopoietic progenitors, platelets, and megakaryocytes (Iancu-Rubin et al., 2014; Mahfoudhi et al., 2016). Preclinical testing of MK-8242 showed that dosing sufficient to trigger a response in vivo achieved drug levels that exceeded those observed at the maximum tolerable dose for adults; therefore, MDM2 inhibition as a treatment strategy may be intolerable for pediatric patients at doses that achieve drug levels required for clinical activity (Kang et al., 2016). Consistent with this hypothesis, MDM2 inhibitor dosing was limited during a previous neuroblastoma trial conducted by Dr. Mosse due to thrombocytopenia. Dr. Scholler suggested that further experimentation to increase understanding of MDM2 inhibitory mechanisms that affect blood cell populations (like megakaryocytes) may provide insights into how to mitigate this toxicity and design more specific molecules that target tumor cells.
Additional Considerations for Preclinical and Clinical Development

After the conclusion of Dr. Adam’s MDM2 presentation, meeting attendees engaged in further discussion about potential MDM2 inhibitor resistance, biomarkers predictive of MDM2 inhibitor success, and MDM2 treatment combinations.

Resistance Mechanisms
Dr. Rosane Charlab Orbach explained that tumors with acquired resistance to MDM2 inhibitors may become more refractory, so understanding mechanisms of resistance is critical to further pursue MDM2 inhibitors as viable therapeutics for pediatric cancers. Potential mechanisms for resistance acquisition include loss of p53 expression/function and high expression of murine double minute X (MDMX). However, Dr. Ronald Bernardi noted that MDM2 amplification minimally impacts sensitivity to MDM2 inhibition. In addition, TP53-independent functional mechanisms of MDM2 require further study to potentially predict and mitigate side effects.

Predictive Biomarkers
Dr. Bernardi suggested identifying biomarkers that predict sensitivity and response to MDM2 inhibitors in order to successfully identify those patients most likely to respond to MDM2 inhibitor therapies. Subject matter experts indicated that loss of p53 expression/function, high MDMX/MDM4 expression, and cyclin-dependent kinase inhibitor 2A deletion may predict MDM2 inhibitor resistance. Dr. Dyer indicated that, in his experience, the best predictor of sensitivity to MDM2 inhibition is sensitivity to ionizing radiation.

Treatment Combinations
Dr. Bernardi recommended combination treatment regimens with drugs activating p53 pathway be considered for MDM2 inhibitors because TP53 pathway activation sensitizes tumors to chemotherapy and other treatments. Using MDM2 inhibitors to sensitize tumors to other therapeutics, rather than as a single agent therapy, may reduce MDM2 inhibitor dosing required to elicit treatment response.

Next Steps
MDM2 is a moderate priority drug target for development in pediatric oncology indications. Because MDM2 inhibitors are generally toxic at higher doses, synergistic targeted combination therapies could be leveraged to maintain therapeutic effects using a reduced MDM2 inhibitor dose. In addition, combination therapies using an MDM2 inhibitor with chemotherapy, such as venetoclax, or radiation could help improve treatment response to chemotherapy or radiation alone (Van Goethem et al., 2017; Vernooij et al., 2021). Companies developing second-generation MDM2 inhibitors should test these therapeutics in preclinical models expected to exhibit MDM2 sensitivity, including wildtype TP53 tumors, neuroblastoma, and hematologic cancers (e.g., leukemias, lymphomas, and multiple myelomas).
CD47
Stacey Adam, PhD, FNIH

Overview
CD47 is a transmembrane immunoglobulin that is normally expressed ubiquitously on cell membranes and prevents the body’s immune system from destroying healthy cells. Tumor cells often overexpress CD47, enabling them to evade phagocytosis by immune cells.

Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival
Researchers and clinicians have observed very few CD47 alterations across pediatric cancer indications, and CD47 gene alterations do not predict high sensitivity to anti-CD47 therapies.

All key pediatric tumor indications exhibit high CD47 expression, with especially high expression in ALL, glioma, neuroblastoma, and possibly pineoblastoma. In addition, T-ALL expresses higher levels of CD47 than B-ALL (Lock et al., 2021).

In Vitro and In Vivo Dependency
Deletion of CD47 has demonstrated no effect on in vitro cell viability across pediatric cancer indications. CD47 surface expression on tumor cells prevents immune cells from destroying those tumor cells; therefore, anti-CD47 mAb therapies block CD47-immune cell interactions and would not reduce in vitro tumor cell lines expression. Any in vivo studies have investigated the dependency for CD47.

In Vitro and In Vivo Drug Sensitivity
No studies conducted to date have assessed in vitro drug sensitivity for CD47 therapies because most anti-CD47 therapeutics are mAbs. However, in vivo drug sensitivity data strongly support the efficacy of anti-CD47 therapies in ALL, glioma, and osteosarcoma (Mohanty et al., 2019).

Additional Considerations for Preclinical and Clinical Development
After the conclusion of Dr. Adam’s CD47 presentation, meeting attendees engaged in further discussion about CD47 treatment combinations, additional CD47 treatment indications, and the effect of signal regulatory protein alpha (SIRPα) expression on CD47 therapeutics.

Treatment Combinations
Chemotherapy—Dr. Zacharoulis explained that although dose timing for anti-CD47 therapies with chemotherapy has not been established, some studies of glioblastoma have demonstrated synergism between anti-CD47 therapies and temozolomide. Dr. Jamie Bates added that for Gilead, the most effective anti-CD47 therapy combination is with azacytidine, a hypomethylating agent.

Immunotherapy—Dr. Smith asked if there was any relevance to this class of agents for treatment of brain cancers, and Dr. Maris explained that while the issues of crossing the blood

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7 Based on pineoblastoma data from only 14 patients
brain barrier (BBB) are ever present, a combination of anti-CD47 therapy with immunotherapy might be useful in reducing bulk of disease in brain cancer patients if the BBB issue can be overcome. Dr. Maris did note this is not a long-term treatment strategy for this disease. Dr. Kieran concurred that if anti-CD47 therapies need to interact directly with tumors and the microenvironment, this therapeutic target may be irrelevant for brain tumors. The only way it might be useful is if the tumor causes leakiness of the BBB. Previous research suggests that nivolumab and CTLA antibodies may “educate” T cells in the periphery to indirectly exert effects on the brain. However, additional CD47 studies in immune-competent in vivo models are required to understand mechanisms for CD47 in brain cancers, and having models that really address the underlying immune biology is key for that.

**Monoclonal Antibodies**—Dr. Jamie Bates explained that historically, researchers determined anti-CD47 treatment works best with other mAbs that recognize tumor cells. Dr. Maris hoped that as additional mAbs to other targets are approved, they would subsequently test those in combination with anti-CD47 mAbs. So far, evidence from pediatric preclinical models supports the development of monoclonal combination therapies with anti-CD47 for pediatric indications. Anti-CD47 antibodies inhibit the CD47-SIRPα checkpoint, resulting in neutrophil-mediated destruction of dinutuximab (i.e., anti-ganglioside GD2 mAb)-opsonized neuroblastoma cells in vitro. Inhibition of CD47 and GD2 with mAbs also demonstrated synergy in treatment of syngeneic and xenograft neuroblastoma mouse models (Martínez-Sanz et al., 2021; Theruvath et al., 2022). An ongoing Phase I clinical trial aims to determine dosing and assess the efficacy of a similar therapeutic combination (i.e., CD47 inhibition with magrolimab and GD2 inhibition with dinutuximab) for neuroblastoma and osteosarcoma in children and young adults (National Cancer Institute, 2022b). Another monoclonal antibody combination involving anti-CD47 monoclonal antibodies and daratumumab (a CD38 monoclonal antibody) potentiated phagocytosis of T-ALL cells in vitro and slowed tumor growth in T-ALL PDX mouse models (Müller et al., 2022). Additional preclinical studies monoclonal antibody combination therapies using immune-competent in vivo models may elucidate mechanisms through which monoclonal antibody therapeutics influence immune system activities.

**Additional Treatment Indications**
Robust clinical development of anti-CD47 therapies is ongoing for cancer indications, including AML, myelodysplastic syndrome (MDS), and Hodgkin’s and non-Hodgkin’s lymphoma, but these data were not included in the current drug target analyses. Therefore, group members should consider including these additional cancer indications.

**Effect of SIRPα Expression**
Dr. Meera Patturajan suggested the level SIRPα expression in macrophages may impact the efficacy of anti-CD47 therapies because CD47 inhibition blocks interactions between macrophage SIRPα and tumor cell CD47. Dr. Zacharoulis noted that SIRPα expression data already exist from in vitro and in vivo models used to test CD47 therapies. However, he cautioned that the role of macrophages in pediatric sarcomas can be unpredictable.
Next Steps
CD47 is a moderate priority drug target for further development in pediatric indications. Preclinical research is needed in sarcoma and ALL models that express CD47 to determine tumor sensitivity to CD47 inhibition. In addition, characterization of SIRPα expression patterns may provide further insight into CD47 inhibitor sensitivity. Furthermore, preclinical testing is needed to identify treatment combinations, especially those with standard chemotherapies.
Appendix A: Feedback

Meeting attendees discussed strategies for improving drug target selection, pre-meeting materials, and executive summary presentations. They also discussed the possibility of a white paper publication including consensus recommendations for drug target development.

Target Selection

Dr. John Maris requested the addition of CD276/B7H3 as another checkpoint drug target for review during the quarterly collaboration meeting on December 14, noting robust preclinical data support consideration of this target.

Dr. Heather Wasserstrom requested that Convening Experts in Oncology to Address Children’s Health (COACH) members vote more frequently on drug targets to better reflect emerging preclinical and clinical study findings. Dr. Stacey Adam will adjust the frequency of target nominations and voting to occur every six months, with the next vote for candidate drug targets scheduled during the March quarterly collaboration meeting.

Pre-Meeting Data Package

Level of Detail

Drs. Maris and Mike Dyer indicated that the level of detail provided in the pre-meeting materials was the appropriate. However, Dr. Vickie Buenger expressed concern that overly scientific slides, and the subsequent groups discussions, may limit the contributions of patient advocates. Therefore, Dr. Adam will schedule a meeting with Dr. Buenger to discuss strategies for making data and discussions more accessible for patient advocates.

Suggested Adjustments

Dr. Martha Donoghue requested that pediatric clinical trial landscape intensity data be separated from that of adult clinical trials. Dr. Olga Almacellas suggested using more restrictive dependency data cutoffs (e.g., Chronos score cutoff between -0.4 and -0.5). Dr. Rosanne Charlab Orbach requested that general safety issues associated with each drug target be included in subsequent slide decks and presentations.

Executive Summary Presentation

Include Additional Context on Target Development

Drs. Wasserstrom and Maris suggested that Dr. Adam add another introduction slide, containing a summary of drug target development, mechanisms of action, and FDA approval statuses, to the executive summary presentation slide decks. The expanded slide decks already contain this information, and Dr. Adam could add the requested information to the executive summary presentations. Dr. Maris also suggested that the commercial intensity slides included in the expanded slide decks be added to the executive summary slide decks.
Recruit Specialized Subject Matter Experts
Dr. Stergios Zacharoulis suggested involving teams of existing COACH members with expertise specific to each target, during the preparation of pre-meeting materials, to suggest additional resources and references. To mitigate potential bias towards individual therapeutic agents, the expert team should include one member with a clinical background and one in academia. The expert team should assist in pre-reading material preparation and present executive summary slides during subsequent meetings.

White Paper
Although FNIH will add a finalized summary of this meeting to the new COACH website, Dr. Hubert Caron strongly recommended that the group consider a more formal publication. After meeting attendees review the meeting summary, they will further discuss interest in drafting a white paper.

Meeting participants agreed that if COACH decides to publish a white paper, the manuscript should be drafted in a timely manner to ensure its relevance. In addition, participants agreed that a white paper would be appropriate only if COACH forms consensus opinions and recommendations for subsequent pediatric oncology drug development activities.
Appendix B: Agenda

10:00 AM  Welcome and Introductions
          Review pre-read materials and discussion of targets
10:15 AM  EZH2 Executive Summary
10:30 AM  EZH2 Discussion
11:15 AM  MDM2 Executive Summary
11:30 AM  MDM2 Discussion
12:15 PM  CD47 Executive Summary
12:30 PM  CD47 Discussion
1:15 PM   Feedback on pre-reading materials and discussion of selection of next targets
2:00 PM   Adjourn
Appendix C: Bibliography


