Convening Experts in Oncology to Address Children’s Health

Quarterly Collaboration Meetings in Pediatric Oncology

June 13, 2023 | Virtual Meeting

Reviewed Targets:
- BCL-2
- MCL-1
- BCL-xL

This meeting summary was prepared by Rose Li and Associates, Inc., under contract to The Foundation for the National Institutes of Health (FNIH). Review of earlier versions of this meeting summary by the following individuals are gratefully acknowledged: Kelly E. Beazley and Gina Castelvecchi.

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<td>ADC</td>
<td>antibody-drug conjugate</td>
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<tr>
<td>AE</td>
<td>adverse event</td>
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<td>ALL</td>
<td>acute lymphocytic leukemia</td>
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<td>AML</td>
<td>acute myeloid leukemia</td>
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<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>B-ALL</td>
<td>B-cell acute lymphocytic leukemia</td>
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<td>B7H3</td>
<td>B7 homolog 3</td>
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<tr>
<td>BAK</td>
<td>B-cell lymphoma 2 antagonist killer</td>
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<td>BAX</td>
<td>B-cell lymphoma 2 associated X</td>
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<tr>
<td>BCL-2</td>
<td>B-cell lymphoma 2</td>
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<td>BCL-xL</td>
<td>B-cell lymphoma-extra large</td>
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<td>BCL-xS</td>
<td>B-cell lymphoma short isoform</td>
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<td>BCP-ALL</td>
<td>B-cell precursor acute lymphoblastic leukemia</td>
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<td>BET</td>
<td>Bromodomain and extra-terminal domain</td>
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<td>BH3</td>
<td>B-cell lymphoma 2 homology 3</td>
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<td>BIM</td>
<td>B-cell lymphoma 2-like 11</td>
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<td>CCSK</td>
<td>clear cell sarcoma of the kidney</td>
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<td>CDX</td>
<td>cell line-derived xenograft</td>
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<td>CLL</td>
<td>chronic lymphocytic leukemia</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CNV</td>
<td>copy number variation</td>
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<td>COACH</td>
<td>Convening Experts in Oncology to Address Children’s Health</td>
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<td>CR</td>
<td>complete remission</td>
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<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>Cy-Topo</td>
<td>cyclophosphamide and topotecan</td>
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<td>DepMap</td>
<td>Dependency Map</td>
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<td>DLBCL</td>
<td>diffuse large B cell lymphoma</td>
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<td>DLT</td>
<td>dose-limiting toxicity</td>
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<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
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<td>EMA</td>
<td>European Medicines Agency</td>
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<td>EwS</td>
<td>Ewing sarcoma</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FFPE</td>
<td>formalin fixed paraffin embedded</td>
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<td>FLT3</td>
<td>feline McDonough sarcoma-related receptor tyrosine kinase 3</td>
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<td>FMS</td>
<td>feline McDonough sarcoma</td>
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<td>HCC</td>
<td>hepatocellular carcinoma</td>
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<td>HEL</td>
<td>human erythroid leukemia</td>
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<td>hiPSC</td>
<td>human induced pluripotent stem cell</td>
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<tr>
<td>HL</td>
<td>Hodgkin’s lymphoma</td>
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<tr>
<td>ITCC-P4</td>
<td>Paediatric Preclinical Proof of Concept Platform</td>
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<tr>
<td>ITD</td>
<td>internal tandem duplication</td>
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<tr>
<td>LBL</td>
<td>lymphoblastic lymphoma</td>
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<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
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<tr>
<td>MCL</td>
<td>mantle cell lymphoma</td>
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<td>myeloid cell leukemia 1</td>
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<td>MDM2</td>
<td>mouse double minute 2</td>
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<td>MDS</td>
<td>myelodysplastic syndrome</td>
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<td>MLL-r</td>
<td>mixed-lineage leukemia rearrangement</td>
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<td>MMTV-LTR</td>
<td>mouse mammary tumor virus long terminal repeat</td>
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<td>MOMP</td>
<td>mitochondrial outer membrane permeabilization</td>
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<td>NCI</td>
<td>National Cancer Institute</td>
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<td>NF1</td>
<td>neurofibromatosis 1</td>
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<td>nHL</td>
<td>non-Hodgkin’s lymphoma</td>
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<td>NIH</td>
<td>National Institutes of Health</td>
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<td>OR</td>
<td>objective response</td>
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<td>ORR</td>
<td>overall response rate</td>
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<td>PD</td>
<td>progressive disease</td>
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<td>PDX</td>
<td>patient-derived xenograft</td>
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<td>PEG</td>
<td>Polyethylene glycol</td>
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<td>PIVOT</td>
<td>Pediatric Preclinical In Vivo Testing</td>
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<td>PMAIP1</td>
<td>phorbol-12-myristate-13-acetate-induced 1</td>
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<td>PPTC</td>
<td>Pediatric Preclinical Testing Consortium</td>
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<td>PRISM</td>
<td>Profiling Relative Inhibition Simultaneously in Mixtures</td>
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<td>PROTAC</td>
<td>proteolysis targeting chimera</td>
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<td>PTPN11</td>
<td>protein tyrosine phosphatase non-receptor type 11</td>
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<tr>
<td>r/r</td>
<td>relapsed or refractory</td>
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<tr>
<td>RAF</td>
<td>rapidly accelerated fibrosarcoma</td>
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<td>RAS</td>
<td>rat sarcoma viral oncogene</td>
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<td>RMS</td>
<td>rhabdomyosarcoma</td>
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<td>RP2D</td>
<td>Recommended Phase II Dose</td>
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<td>SD</td>
<td>stable disease</td>
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<td>siRNA</td>
<td>small interfering RNA</td>
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<td>SLL</td>
<td>small lymphocytic leukemia</td>
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<td>SME</td>
<td>subject matter expert</td>
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<td>SSM</td>
<td>simple somatic mutation</td>
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<td>SV</td>
<td>structural variations</td>
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<td>T-ALL</td>
<td>T-cell ALL</td>
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<td>TME</td>
<td>tumor microenvironment</td>
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<td>TNF</td>
<td>tumor necrosis factor</td>
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<td>TRAIL-R</td>
<td>tumor necrosis factor-related apoptosis-inducing ligand receptor</td>
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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 June 13, 2022, COACH convened the Fifth 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: B-cell lymphoma 2 (BCL-2), myeloid cell leukemia 1 (MCL-1), and B-cell lymphoma-extra-large (BCL-xL).

BCL-2

BCL-2 inhibits the mitochondrial-dependent apoptosis pathway by preventing BCL-2 associated X (BAX) and BCL-2 antagonist killer (BAK) oligomerization. Increased BCL-2 expression can inhibit apoptosis and facilitate tumor development and resistance to apoptotic signals. While mutations in BCL-2 are not cancer drivers, BCL-2 expression has a mixed association with patient outcomes in gliomas, neuroblastomas, and osteosarcomas. Current in vitro data support BCL-2 as a promising target in leukemias and neuroblastomas, with limited single-agent potential in other tumor types. Venetoclax, a BCL-2 inhibitor, has shown single-agent efficacy in acute lymphocytic leukemia (ALL) models, as well as additive anti-tumor effects in combination therapies that promote apoptosis, while single-agent activity in acute myeloid leukemia (AML) in vivo models was variable. BCL-2 studies in solid tumor models are limited, with potential solid tumor applications for BCL-2 inhibition in MYCN-amplified neuroblastomas and non-Hodgkin’s lymphoma (NHL). However, use of BCL-2 inhibitors requires careful consideration for adverse events (AEs) related to dramatic lymphocytopenia and mild anemia.

Currently, venetoclax is FDA-approved for two adult indications and in Phase III clinical trials for three other adult indications, and venetoclax is also being tested in pediatric indications. Although venetoclax is the only FDA-approved BCL-2 inhibitor, 18 BCL-2 inhibitors are in development, of which 10 are in Phase II or III clinical trials.

Key Considerations

• Use of BCL-2 inhibitors requires strategies to mitigate lymphocytopenia and anemia, especially when used in conjunction with other therapies with hematological toxicities.
Additional strategies may include discontinuous dosing schedules, reduced BCL-2 inhibitor dosing, and more targeted BCL-2 inhibition using proteolysis targeting chimeras (PROTACs) or antibody-drug conjugates (ADCs).

- BCL-2 inhibition has notably strong single-agent activity in preclinical ALL models, but current pediatric clinical trials indicate a lack of activity in patients with relapsed or refractory (r/r) ALL. BCL-2 inhibitors likely will require administration in combination with other pro-apoptotic therapies for efficacy in various tumor types.
- Effects of BCL-2 inhibitors may be attenuated in tumors that have previously been exposed to multiple pro-apoptotic therapies due to attenuated mitochondrial functions.
- Additional biomarkers feasible for clinical use are needed to predict which tumors will be sensitive to BCL-2 inhibitors. BCL-2 expression itself does not predict BCL-2 inhibitor sensitivity, and while BCL-2-B-cell lymphoma 2-like 11 (BIM) interaction with BCL-2 predicts BCL-2 inhibitor sensitivity, current protein-protein interaction assays are not feasible for clinical use.

MCL-1
Similar to BCL-2, MCL-1 is an anti-apoptotic protein; it sequesters BAX and BAK to prevent apoptosis and also has roles in maintaining mitochondrial homeostasis. While alterations in MCL-1 are rare across pediatric tumors, it is highly expressed across pediatric tumor types. Current in vitro data support targeting MCL-1 in multiple solid tumors and blood cancers with moderate to high MCL-1 dependency. Strong MCL-1 inhibitor activity has been reported in AML preclinical models, and combined inhibition of MCL-1 and other BCL-2 family proteins has promising anti-tumor effects. Importantly, MCL-1 is required for T and B cell survival, as well as mitochondrial function in cardiomyocytes; therefore, dosing strategies and monitoring are required for effective use of MCL-1 inhibitors.

There are no FDA-approved MCL-1 inhibitors, and 8 inhibitors are currently in development. None of these MCL-1 inhibitors have been tested in pediatric populations.

Key Considerations
- Preclinically, MCL-1 inhibition exhibited strong single-agent effects in AML models and strong effects in B-ALL models when administered with venetoclax. Certain neuroblastoma preclinical models strongly respond to MCL-1 inhibition as well.
- MCL-1 inhibitors are associated with hematological and cardiac toxicities. To mitigate these effects, adjustments to treatment strategies may be required.
  - Discontinuous dosing schedules and dose optimization may help mitigate these toxicities, especially when used in combination with other agents that have overlapping hematological and cardiac toxicities.
  - More targeted inhibition of MCL-1 via PROTACs or ADCs may also reduce toxicities.
- No MCL-1 inhibitors have been clinically tested in pediatric populations.
- MCL-1 inhibitors likely will require co-administration with a BCL-xL inhibitor due to functional redundancies. In MCL-1-dependent tumors exposed to MCL-1 inhibitors,
rather than experiencing anti-apoptotic effects, BIM switches its binding from MCL-1 to BCL-xL, preventing apoptosis. These same tumors are often sensitive to dual inhibition of MCL-1 and BCL-xL.

- Effects of MCL-1 inhibitors may be attenuated in tumors previously exposed to multiple pro-apoptotic therapies due to attenuated mitochondrial functions.
- Biomarkers feasible for clinical use are needed to predict which tumors will be sensitive to MCL-1 inhibitors. MCL-1 expression alone does not predict sensitivity, and although the MCL-1-BIM interaction predicts BCL-2 inhibitor sensitivity, current protein-protein interaction assays are not feasible for clinical use.

**BCL-xL**

Similar to BCL-2 and MCL-1, BCL-xL exerts its anti-apoptotic effects by inhibiting BAX and BAK oligomerization. BCL-xL overexpression is a key driver of tumors with BCL-xL perturbations, although BCL-xL is expressed only at low levels in most pediatric cancer cell lines. Navitoclax, a dual inhibitor of BCL-2 and BCL-xL, has shown single-agent activity in ALL and mixed activity among different AML preclinical models. Based on existing preclinical data, navitoclax has poor activity in solid tumors, but BCL-xL studies in solid tumors are lacking overall. The most notable toxicity associated with BCL-xL inhibition is thrombocytopenia.

There are no FDA-approved BCL-xL inhibitors. Navitoclax is the only agent that inhibits BCL-xL currently being tested in pediatric populations, in combinations that include venetoclax and/or chemotherapy in AML, ALL, and nHL.

**Key Considerations**

- Because most preclinical and clinical data on BCL-xL inhibition is from studies of navitoclax, a dual BCL-xL and BCL-2 inhibitor, navitoclax efficacy cannot be ascribed solely to BCL-xL inhibition.
- Multiple ALL preclinical models are highly sensitive to single-agent navitoclax, and co-administration of navitoclax and venetoclax sometimes results in synergistic effects in ALL. Because navitoclax already inhibits BCL-2, the contribution of venetoclax to this synergism remains poorly understood.
- Navitoclax and other BCL-xL inhibitors have limited single-agent activity in preclinical solid tumor models but may be effective in neuroblastoma when administered with chemotherapy.
- BCL-xL inhibitors are associated with severe thrombocytopenia that requires mitigation either by dosing strategies or by more targeted inhibition via PROTACs and ADCs, especially when administered with other agents associated with thrombocytopenia. The strategy of using a BCL-xL degrader that utilizes a E3-ubiquitin ligase that is expressed at low levels in platelets is under clinical evaluation.
- Navitoclax is the only BCL-xL inhibitor currently being tested in clinical trials of pediatric populations with various leukemias. No results are currently available.
- The effects of BCL-xL inhibitors may be attenuated in tumors previously exposed to multiple pro-apoptotic therapies due to attenuated mitochondrial functions.
Next Steps

- BCL-2 family proteins are of moderate to high priority for continued preclinical exploration; BCL-2 is of moderate priority and MCL-1 and BCL-xL are moderate-to-high priority.
- Further preclinical development is required to mitigate toxicities associated with BCL-2 family inhibitors. This preclinical research should include optimization of dosing schedules as well as the development of more targeted therapeutics, such as PROTACs and ADCs.
- Robust biomarkers feasible for clinical use are needed to predict tumor sensitivities to BCL-2 family protein inhibitors.
- Further preclinical efficacy studies are needed to identify rational therapy combinations for use with BCL-2 protein family inhibitors. These studies require more diverse pediatric tumor models with different survival dependencies.
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—B-cell lymphoma 2 (BCL-2), myeloid cell leukemia 1 (MCL-1), and B-cell lymphoma-extra-large (BCL-xL)—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.

**BCL-2**

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

**Overview**

BCL-2 inhibits mitochondrial-dependent apoptosis by binding to the BH3 domains of BCL-2 associated X (BAX) and BCL-2 antagonistic killer (BAK), which prevents their oligomerization. Normally, BAX/BAK oligomerization promotes mitochondrial outer membrane permeabilization (MOMP) and subsequent cytochrome c release, activating a caspase-dependent apoptotic cascade. BCL-2 and other BCL-2 family proteins are also important for cellular calcium regulation, mitochondrial dynamics, neuronal activity, and autophagy.

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

BCL-2 alterations are not drivers of pediatric cancer and are detected at low rates in pediatric tumors. Adults with non-Hodgkin’s lymphoma (NHL) have high rates of BCL-2 amplification due to t(14:18) translocations, but these translocations are rare in pediatric NHL cases (Galteland et al., 2005).

Notably, BCL-2 expression is enriched in nervous system tumors and acute lymphocytic leukemias (ALL), but its expression has mixed associations with patient outcomes. High BCL-2 expression in MYC-amplified neuroblastoma is associated with unfavorable tumor histology, while BCL-2 expression is positively correlated with survival in clear cell sarcoma of the kidney (CCSK) and osteosarcoma (Castle et al., 1993). BCL-2 expression is also positively correlated with survival in glioma, but a separate study correlated its expression with poor outcomes in glioblastoma (Ganigi et al., 2005).

**Dependency and Drug Sensitivity**

**In Vitro Dependency and Sensitivity**

*In vitro* data indicate that BCL-2 may be a promising drug target in leukemias and neuroblastomas. In agreement with patient tumor expression data, leukemia and central nervous system (CNS) tumor cell lines have enriched expression of BCL-2, but its expression levels vary highly across other tumor cell lines. In non-CNS solid tumor cell lines—especially...
malignant rhabdoid tumor and hepatoblastoma lines—BCL-2 is expressed at low levels. Moreover, most solid tumor cell lines show limited dependency on BCL-2, whereas leukemia cell lines are highly sensitive to BCL-2 deletion (Soderquist et al., 2018). Of the tumor cell lines included in the PRISM cell line screen, the AML lines (i.e., MOLM13 and MV4-11) were sensitive to venetoclax. Overexpression of BCL-xL—another BCL-2 family protein with similar anti-apoptotic functions and interactions with BAK and BAX—in AML lines resulted in venetoclax resistance (Zhang et al., 2020). In addition, neuroblastoma lines with high BCL-2 expression are sensitive to BCL-2 inhibition (Lamers et al., 2012).

**Single-Agent In Vivo Sensitivity**

**Leukemias**

Venetoclax has been extensively studied as a monotherapy and shows additive anti-tumorigenic effects when combined with other anticancer agents in in vivo pediatric ALL models. Daily dosing of pediatric ALL xenografts with venetoclax resulted in mild antitumor activity associated with BCL-xL expression (Khaw et al., 2016). The overall response rate (ORR) for venetoclax in ALL was 29 percent lower than navitoclax (i.e., dual BCL-2 and BCL-xL inhibitor). Chronic lymphocytic leukemia (CLL), myeloid/lymphoid or mixed-lineage leukemia rearrangement (MLL-r) ALL, and hypodiploid B cell ALL (B-ALL) in vivo models also showed venetoclax sensitivity (Diaz-Flores et al., 2019; Seyfried et al., 2019; Suryani et al., 2014).

Pediatric acute myeloid leukemia (AML) in vivo models have shown mixed responses to venetoclax, usually limited to reduced tumor progression. Daily administration of venetoclax alone did not significantly delay tumor progression across multiple AML models overall, although BH3 profiling and JQ-1 (i.e., a bromodomain and extra-terminal domain [BET] inhibitor) resistance can each predict venetoclax sensitivity (Lin et al., 2020; Pan et al., 2014; Tahir et al., 2023). A novel polyethylene glycol (PEG)-conjugated formulation of venetoclax shows improved pharmacokinetics and tumor tissue absorption. In AML models, this PEGylated formulation demonstrated comparable activity to aqueous venetoclax at a lower dose (Ando et al., 2022).

**Other Pediatric Indications**

Preclinical efficacy of BCL-2 inhibitors in pediatric indications outside of leukemias remains poorly understood, although BCL-2 inhibition may be effective in NHL and MYCN-amplified neuroblastoma subsets. High BCL-2 expression in mantle cell lymphoma (MCL) and diffuse large B cell lymphoma (DLBCL) patient-derived xenograft models can predict tumor growth suppression by venetoclax monotherapy (Pham et al., 2018). In addition, a small interfering RNA (siRNA) targeting BCL-2 conjugated to a GD-2 antibody showed modest suppression of tumor growth in an SK-N-SH neuroblastoma cell line-derived xenograft (CDX) (Shen et al., 2012).
In Vivo Sensitivity to Venetoclax Combinations

Leukemias

The following venetoclax combinations have demonstrated enhanced efficacy in preclinical ALL models compared to venetoclax monotherapy:

- Venetoclax plus tyrosine kinase inhibitors (ruxolitinib or dasatinib) in Philadelphia chromosome-like ALL (Ding et al., 2021)
- Venetoclax plus BET inhibitor (JQ-1) in T-ALL (Peirs et al., 2017)
- Venetoclax plus MCL-1 inhibitor (S63845) in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) (Seyfried et al., 2022)

The following venetoclax combinations have demonstrated enhanced efficacy in preclinical AML models compared to venetoclax monotherapy:

- Venetoclax plus feline McDonough sarcoma (FMS)-related receptor tyrosine kinase 3 (FLT3) inhibitor (gilteritinib) in venetoclax-azacitidine-resistant AML (Janssen et al., 2022)
- Venetoclax plus MCL-1 inhibitor (S63845) in BH3 mimetic-resistant AML (Bhatt et al., 2020)
- Venetoclax plus HDAC inhibitors in FLT3-internal tandem duplication (ITD) AML (Chen et al., 2020)
- Venetoclax plus tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor (TRAIL-R) agonist (Eftoza) in multiple AML lines, including those with FLT3-ITD or TP53 mutations (Tahir et al., 2023)

Other Pediatric Indications

Two venetoclax combinations have demonstrated enhanced efficacy in preclinical neuroblastoma models compared to venetoclax monotherapy: (1) venetoclax plus aurora kinase A inhibitor in MYCN-amplified neuroblastoma PDXs and (2) venetoclax plus H3K27 demethylase inhibition in LANS and SK-N-DZ neuroblastoma CDXs (Ham et al., 2016; Lochmann et al., 2018). Notably, MYCN amplification in neuroblastoma PDXs is associated with low BCL-xL expression and high pro-apoptotic NOXA* (encoded by phorbol-12-myristate-13-acetate-induced 1 [PMAIP1]) expression, which likely increase sensitivity to BCL-2 inhibition.

In addition, a combination treatment with venetoclax and an anti-CD20 antibody (rituximab) resulted in synergistic antitumor activity and enhanced long-term survival in DLBCL PDXs with high BCL-2 expression (Vogiatzi et al., 2022).

Preclinical Safety and Toxicology

Previous in vivo studies have examined the effects of loss of BCL-2 on lymphocytes, red blood cells, reproductive organs, and body organs including kidneys and heart. Genetic loss or

* Named from the Latin term noxa, meaning “harm, damage”
pharmaceutical inhibition of BCL-2 results in lymphocytopenia via accelerated apoptosis and mild anemia, although differences in toxicology between rodent and canine models have been reported. In chimeric BCL-2 knockout mice, bone marrow, thymus, and blood lacked T and B cells at four weeks of age (Nakayama et al., 1993). This decrease in lymphocytes is dose-responsive to BCL-2 inhibition and can result in up to 75 percent reduction in lymphocytes following repeat dosing in rodent and canine models, although reversal of this occurred 4 weeks and 18 weeks after the final dose in rodents and canines, respectively. Notably, reductions in blood cell mass were minimal with no differences observed for rodents and canines (Center for Drug Evaluation and Research, 2016; Committee for Medicinal Products for Human Use, 2016).

Studies in male and female mice demonstrated no effects of venetoclax on estrus cycles, mating, or fertility, while in canines, venetoclax caused adverse, non-dose-related microscopic findings in testes. Four weeks after venetoclax dosing, canine testes exhibited severe decreases in spermatogonia and eventually reduction of all germ cells (Center for Drug Evaluation and Research, 2016; Committee for Medicinal Products for Human Use, 2016).

While loss of BCL-2 does not impede embryonic development in mice, it does result in postnatal growth retardation, smaller ears, atrophic thymus and spleen, and defects in gamma motor neurons in the lumbar spinal cord (Hui et al., 2008; Kamada et al., 1995). BCL-2 knockout mice also have shorter lifespans, often caused by polycystic kidney disease (Kamada et al., 1995). In addition, high doses of venetoclax resulted in cardiac histopathological changes, although no cardiac toxicities have been reported in mice and canines (AlAsmari et al., 2022; Committee for Medicinal Products for Human Use, 2016).

Based on studies of venetoclax treatment of in vitro mouse chondrocytes, ex vivo cultures of rat metatarsals, ex vivo cultures of human growth plates, and 6-week-old mice, BCL-2 inhibition suppresses bone growth, likely by directly targeting growth plate chondrocytes. Therefore, BCL-2 inhibitors should be used with caution in children, as they may impede growth (Velentza et al., 2023).

**Clinical Trial Development**

**Adult Clinical Trials**

Numerous adult clinical trials of venetoclax identified significant single-agent antitumor activity in leukemias, with enhanced efficacy when combined with chemotherapy. However, venetoclax also enhances chemotherapy toxicities, with increased frequencies of nausea, vomiting, and diarrhea, as well as grade 3 or higher adverse events (AEs), including febrile neutropenia, anemia, and pneumonia. AEs compared to chemotherapy alone. More specifically, in a Phase I study of adult AML, 5 of 12 patients receiving 1,200 mg venetoclax with chemotherapy required dose reductions to 800 mg due to increased hematological and gastrointestinal adverse events (DiNardo et al., 2019). Therefore, further studies are required to optimize efficacy while mitigating toxicities.
The FDA has approved venetoclax use in adults for the treatment of: (1) CLL/small lymphocytic leukemia (SLL) as a single agent or in combination with rituximab and (2) AML in combination with azacitidine, decitabine, or low-dose cytarabine in patients 75 years of age and older (AbbVie, 2021, 2022a, 2022b, 2023b; Center for Drug Evaluation and, 2019; DiNardo et al., 2019; Griffioen et al., 2022; Hoffmann-La Roche, 2022; *VENCLEXTA® (Venetoclax Tablets) for Oral Use*, 2019). In addition, multiple venetoclax combinations are in Phase III clinical trials: (1) venetoclax plus azacitidine for myelodysplastic syndrome (MDS); (2) venetoclax plus dexamethasone with or without bortezomib for multiple myeloma; and (3) venetoclax plus ibrutinib for MCL (AbbVie, 2022b, 2023c, 2023d; Pharmacyclics LLC., 2023).

**Pediatric Clinical Trials**

AbbVie is currently sponsoring a Phase I clinical trial of venetoclax with and without various chemotherapies to assess dose-limiting toxicities (DLTs) and efficacy in patients 25 years of age and younger with relapsed or refractory (r/r) malignancies as well as determine a Recommended Phase II Dose (RP2D) (AbbVie, 2023a). Venetoclax monotherapy was generally ineffective in pediatric r/r leukemias. Venetoclax plus chemotherapy was well-tolerated in r/r AML and ALL patients (Karol, 2020; Place, 2020). Treatment combinations of r/r AML patients with venetoclax plus azacitidine or high-dose cytarabine resulted in ORRs of 26 percent and 50 percent, respectively, while venetoclax plus decitabine or low-dose cytarabine resulted in no detectable responses (Karol, 2020). Venetoclax plus a combination of dexamethasone and/or vincristine and/or PEG-asparaginase resulted in an ORR of 56 percent in r/r ALL (Place, 2020).

Venetoclax monotherapy of solid tumors in seven pediatric patients achieved a best response of stable disease (SD). In addition, six of seven solid tumor pediatric patients responded to treatment with venetoclax plus cyclophosphamide and topotecan (Cy-Topo) (i.e., one patient with complete response [CR], one patient with progressive disease [PD], and four patients with SD). However, continuous dosing of venetoclax plus Cy-Topo was not well-tolerated in these patients with cytopenia reported in four patients (Goldsmith et al., 2020). Therefore, discontinuous dosing of this treatment combination is being explored as a regimen to maximize efficacy and mitigate toxicities.

**Research and Development Landscape**

Published scientific literature on BCL-2 has remained relatively high with a spike in interest in 2022. Approximately half of publications about BCL-2 are oncology-related, indicating moderate interest in BCL-2 as an oncology target. However, less than one percent of these articles are related to pediatric oncology. All relevant indications except for CCSK are referenced in at least one BCL-2 publication, with especially frequent references to glioma, AML, neuroblastoma, Hodgkin’s lymphoma (HL), NHL, osteosarcoma, and retinoblastoma.

Venetoclax is the only FDA-approved BCL-2 inhibitor, and the development pipeline consists of 18 drugs with 10 of these in Phase II clinical trials and beyond.
MCL-1

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

**Overview**

MCL-1 is a BCL-2 protein family member that also sequesters BAX and BAK proteins via their BH3 domains to prevent apoptosis. Notably, MCL-1 is specifically inhibited by NOXA, and this interaction frees BAX and BAK to initiate MOMP and subsequent caspase-dependent apoptosis. MCL-1 is also involved in other processes related to mitochondrial homeostasis (Perciavalle et al., 2012). MCL-1 is a promising oncology drug target and even underpins some resistance mechanisms to multiple oncology therapies.

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

MCL-1 is highly expressed in pediatric malignancies—especially in AML, glioma, and neuroblastoma—but alterations are infrequent across pediatric cancers and not considered relevant when predicting sensitivity to MCL-1 inhibition. MCL-1 alteration rates are highest in osteosarcomas and Wilms’ tumors, and MCL-1 amplifications have been reported in pediatric osteosarcoma patients (Suehara et al., 2019). In addition, limited evidence supports a correlation between MCL-1 expression and survival in neuroblastoma.

**Dependency and Drug Sensitivity**

*In Vitro Dependency and Sensitivity*

Based on *in vitro* expression, dependency, and sensitivity data, MCL-1 is a promising drug target for the treatment of multiple pediatric oncology indications. Overexpression of MCL-1 has been reported in AML, multiple myeloma, and NHL, and a previous study showed an upregulation of MCL-1 expression in osteosarcoma post-chemotherapy treatment (Liu et al., 2019; Wei et al., 2020). Most tumor types are moderately to highly dependent on MCL-1, and high expression of MCL-1 in blood cancers may be indicative of high dependency (Bolomsky et al., 2020). While the PRISM cell line screen did not include tumor types with expected vulnerability to MCL-1 inhibition, TW-37 (a BCL-2/MCL-1 dual inhibitor) reduced viability of neuroblastoma cell lines, especially those with MYCN amplification (Klenke et al., 2019).

*In Vivo Sensitivity*

**Leukemias**

MCL-1 inhibitors alone, and in combination with BCL-2 inhibitors, show strong antitumor activity in *in vivo* models of AML. A-1210477 alone significantly delayed tumor progression in multiple AML CDXs (MOLM-13, HL-60, MV4-11, OCI-AML3), and AZD5991 resulted in complete regression of MV4-11 CDXs (Tron et al., 2018; Q. Wang & Hao, 2019). AMG-176 plus various chemotherapy regimens resulted in strong synergistic treatment effects in MOLM-13 CDXs (Caenepeel et al., 2018).
When combined with venetoclax, the following MCL-1 inhibitors resulted in significantly better treatment responses in AML, compared to MCL-1 inhibition alone:

- S63845 in venetoclax- or MCL-1 inhibition-resistant PDXs (Bhatt et al., 2020)
- AMG-176 in MOLM-13 CDXs (Caenepeel et al., 2018)
- AZD5991 in OCI-AML3 CDXs with limited response to BCL-2 inhibition alone (Tron et al., 2018)

Preliminary in vivo data suggests that MCL-1 inhibition may also be effective in combination with venetoclax in ALL. The MCL-1 inhibitor S63845 plus venetoclax significantly reduced primary B-ALL burden compared to S63845 or venetoclax alone (Moujalled et al., 2020).

**Solid Tumors**

While few solid tumor studies have tested MCL-1 inhibitors, preliminary evidence suggests that targeting MCL-1 may be effective in neuroblastoma and rhabdomyosarcoma (RMS). Inhibition of MCL-1 with S63845 or TW-37 significantly reduced tumor size in a neuroblastoma CDX (COG-N-415x) and significantly delayed tumor growth in the Kelly cell neuroblastoma model, respectively (Dalton et al., 2021; Klenke et al., 2019). In RMS PDXs, combinations of S63845 plus vincristine or trametinib inhibited tumor growth but did not result in remission (Alcon et al., 2020, 2022).

**Preclinical Safety and Toxicology**

Genetic loss or pharmacological inhibition of MCL-1 results in deficits in immune cell survival similar to loss of BCL-2 as well as cardiac function. Conditional loss of MCL-1 in mice led to greater than an 80 percent reduction in T and B lymphocytes as a result of increased apoptosis (Opferman et al., 2003). Conditional loss of MCL-1 in bone marrow also results in severe anemia and loss of hematopoietic stem cells (Opferman et al., 2005). Consistent with genetic studies, inhibition of MCL-1 with AMG-176 leads to reductions of B cell and monocyte populations with no effect on neutrophil levels (Caenepeel et al., 2018).

Cardiac-specific loss of MCL-1 results in fatal dilated cardiomyopathy due to loss of cardiac contractility and abnormal mitochondrial structure and function. This defect is independent from embryonic development and not due to an increase in apoptosis (Thomas et al., 2013; X. Wang et al., 2013). The apoptosis-independent nature of this cardiac defect was also confirmed in a human induced pluripotent stem cell (hiPSC) cardiomyocyte model (Rasmussen et al., 2020).

Notably, combined heterozygosity of MCL-1 and BCL-xL results in reduced organ size, more prominent in males than females (Ke et al., 2020).

**Clinical Trial Development**

There are no FDA-approved MCL-1 inhibitors, and MCL-1 inhibitors have not entered pediatric clinical trials. However, MCL-1 inhibitors are being tested in 6 clinical trials for adult cancers, which may provide further insights relevant for future pediatric cancer testing.
Research and Development Landscape
Based on the amount of MCL-1 literature focused on oncology indications, MCL-1 has been consistently studied by the scientific community; however, only one percent of all MCL-1 publications are related to pediatric oncology. Frequently referenced indications include AML, glioma, and ALL, while hepatoblastoma, ependymoma, pineoblastoma, and CCSK are not referenced in any MCL-1 publications.

No MCL-1 inhibitors are FDA-approved, but eight inhibitors are in development: AMG-176 (Amgen), CT-03 (Captor Therapeutics), fadraciclib (Cyclacel), FL-118 (Canget BiotekPharma), GC-9716 (Gilead), PRT-1419 (Prelude Therapeutics), S-64315 (Servier), and STP-369 (Sirnaomics). Despite efficacy in some AML CDXs, AstraZeneca no longer lists AZD5991 as part of its oncology program and therefore likely discontinued development.

BCL-xL
Stacey Adam, PhD, Foundation for the National Institutes of Health

Overview
BCL-xL inhibits apoptosis similarly to BCL-2 and MCL-1: by inhibiting oligomerization of BAX and BAK. BCL-xL is also important for survival of erythroid lineage cell types (Mason et al., 2007). BCL-xL is encoded by BCL2L1, which contains a long isoform (BCL-xL) and a short isoform (BCL-xS). BCL-xL, the main isoform, is anti-apoptotic, while BCL-xS, the minor isoform, has pro-apoptotic functions. Both dysregulation of BCL2L1 gene splicing and overexpression of BCL-xL are broadly implicated in cancer (Dou et al., 2021).

Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival
BCL-xL is overexpressed across cancer types and promotes tumor progression (Trisciuoglio et al., 2017). BCL2L1 alterations are rare in pediatric cancers, and BCL2L1 splicing is not highly dependent on mutations (Dou et al., 2021). Relevance of BCL-xL expression levels to survival rates remains unclear. In patients with osteosarcoma, prior studies have reported negative correlations between BCL-xL expression and survival, while high BCL-xL expression in AML is associated with chemoresistance and poorer patient outcomes (Konopleva et al., 2002; Z.-X. Wang et al., 2010).

Dependency and Drug Sensitivity

In Vitro Dependency and Sensitivity
Despite low levels of BCL-xL expression in most pediatric cancer cell lines, these same cell lines show moderate to high dependency on BCL-xL. The moderate level of BCL-xL in some ALL cell lines is both independent of p53 and associated with radiotherapy resistance (Findley et al., 1997). Based on siRNA studies, pediatric osteosarcoma, neuroblastoma, RMS, and Ewing sarcoma (EwS) cell lines are actually co-dependent on BCL2L1 and MCL-1 for survival (Kehr et al., 2020).
While the PRISM cell line screen identified modest sensitivity of multiple cancer cell lines to navitoclax (a dual inhibitor of BCL-2 and BCL-xL), *in vitro* data related to pediatric cell lines is scarce. Navitoclax may have therapeutic potential for medulloblastoma based on its activity in medulloblastoma cell lines and artificial tumor models (Westhoff et al., 2022). Moreover, prior studies demonstrated that pediatric osteosarcoma, neuroblastoma, RMS, and EwS cell lines are resistant to BCL-xL inhibition alone but are highly susceptible to combined BCL-xL and MCL-1 inhibition. Additional siRNA studies confirmed this BCL-xL/MCL-1 co-dependency for survival (Kehr et al., 2020).

**In Vivo Sensitivity**

**Leukemias**

Navitoclax has strong single agent activity in preclinical *in vivo* ALL models, while mixed results of its single agent activity have been reported for AML. In a study of navitoclax in 31 pediatric ALL PDXs, 19 demonstrated objective responses (ORs), and 7 of 14 T-ALL PDXs achieved CRs (Suryani et al., 2014); additional studies have also found that T-ALL xenografts are highly sensitive to navitoclax (Lock et al., 2008). Moreover, T-ALL models accumulate less resistance to navitoclax compared to venetoclax (Chonghaile et al., 2014). Notably, navitoclax plus venetoclax had marked synergistic activity in 7 of 24 ALL xenografts, although the mechanism for this synergism remains poorly understood (Khaw et al., 2016).

The BCL-xL inhibitor A-1155463 demonstrated mixed anti-tumor effects in AML CDXs. No activity was detected in HL-60, MOLM-13, THP-1, and OCI-AML3, while mild activity was only detected in U937 and KG-1 (Q. Wang et al., 2019). High-throughput screening detected sensitivity to A-1331852 treatment in erythroid and megakaryocytic AMLs that also highly express BCL-xL, and this inhibitor showed strong activity in human erythroid leukemia (HEL) xenograft models (Kuusanmäki et al., 2023).

**Solid Tumors**

Navitoclax exhibits limited single-agent activity in solid tumor models. In addition, few effective combination therapies for solid tumors have been identified that include navitoclax (Lock et al., 2008). ABT-737 plus cyclophosphamide exhibits strong activity in BCL-2-dependent neuroblastoma lines, while a BCL-xL proteolysis targeting chimera (PROTAC) therapy (DT2216) plus irinotecan showed activity in fibrolamellar hepatocellular carcinoma (HCC) models (Goldsmith et al., 2012; Shebl et al., 2022).

**Preclinical Safety and Toxicology**

Genetic loss and pharmaceutical inhibition of BCL-xL results in thrombocytopenia and anemia. BCL-xL knockout mice exhibit apoptosis in hematopoietic tissues and BCL-xL lymphocyte chimeras showed diminished lymphocyte maturation. These hematopoietic defects in BCL-xL knockouts, paired with increased apoptosis in postmitotic immature neurons and in the liver, result in lethality at embryonic day 13 (Motoyama et al., 1995).
Platelet survival and red blood cell homeostasis also require functional BCL-xL. Genetic loss or pharmacological inhibition of BCL-xL in mice reduces platelet half-life and results in thrombocytopenia in a dose-dependent manner, and these toxicities are also reflected in subsequent clinical trials (Mason et al., 2007; Wilson et al., 2010). After a single dose of ABT-737 in mice, platelet counts reduced by 30 percent but gradually recovered to normal levels three days post-treatment (Mason et al., 2007).

BCL-xL pharmacological inhibition causes a significant loss of erythroid cells and reduction of megakaryocytes (Afreen et al., 2020). Conditional loss of BCL-xL in erythroid cells of mice (achieved via the Cre-loxP system under control of mouse mammary tumor virus long terminal repeat [MMTV-LTR]) also resulted in hemolytic anemia; however, this toxicity was accompanied by hyperplasia of erythroid cells and spleen enlargement (Wagner et al., 2000). One potential explanation for the seemingly opposing findings of reduced erythroid cells and erythroid cell hyperplasia in the spleen is that apoptosis of erythroid cells occurs at late maturation stages, triggering increased proliferation of immature erythroid cells to compensate (Wagner et al., 2000).

Loss of a single copy of BCL-xL in mice results in fertility and hepatic defects. BCL-xL heterozygous mice exhibit increased apoptosis in male germ cells around embryonic day 13.5, resulting in reduced fertility (Kasai et al., 2003). In addition, these heterozygous mice are more susceptible to hepatic injury in binge alcohol abuse and TNF-α-induced injury models (Henderson et al., 2005).

**Clinical Trial Development**

Thrombocytopenia is a common toxicity seen in adult clinical trials of navitoclax and can be dose-limiting. Based on one adult study, a 150 mg 7-day lead-in dose of navitoclax followed by a 325 mg dose on a continuous 21-day schedule was selected as the dosing schedule for Phase II clinical trials.

Navitoclax is the only BCL-xL inhibitor being evaluated in pediatric clinical trials. A Phase I/II clinical trial (NCT05740449) for patients 1 to 21 years of age with r/r ALL or lymphoblastic lymphoma (LBL; a type of NHL) will test the safety and efficacy of navitoclax plus decitabine plus venetoclax (Princess Maxima Center for Pediatric Oncology, 2023). Another Phase I clinical study (NCT05222984) of the same treatment combination is currently recruiting patients 16 years of age and older with r/r AML after previous venetoclax treatment to evaluate AEs and determine the optimal dose for navitoclax (City of Hope Medical Center, 2023). In addition, to evaluate minimal residual disease and identify an RP2D for navitoclax, a Phase I/II study of navitoclax plus venetoclax plus chemotherapy (NCT05192889) is currently recruiting patients between the ages of 4 and 30 years with r/r ALL (St. Jude Children’s Research Hospital, 2023).

**Research and Development Landscape**

As evidenced by the number of BCL-xL research articles related to oncology, BCL-xL is likely an oncology drug target of moderate interest to the research community. However, only one percent of all BCL-xL publications are studies in pediatric oncology. Oncology BCL-xL articles
most frequently reference glioma, AML, and neuroblastoma, but not rhabdoid tumors, pineoblastoma, and CCSK. There are no FDA-approved drugs that target BCL-xL, although eight agents are currently in development: navitoclax (AbbVie), ABBV-637 (AbbVie), pelcitoclax (Ascentage Pharma), AZD-0466 (AstraZeneca), a BCL-xL degrader (Zentalis), DT-2216 (Dialectic Therapeutics), NWP-4-76 (Newave Pharmaceutical), STP-369 (Sirnaomics), UBX-1325 (Unity Biotechnology), and two BCL-xL degraders (one being developed by Zentalis and the other, DT-2216, being developed by Dialectic Therapeutics).

Additional Considerations for Preclinical and Clinical Development  
_Facilitated by Kelly Goldsmith, MD, Emory University, and Michael Hogarty, MD, Children’s Hospital of Philadelphia_

After the conclusion of Dr. Adam’s presentations on BCL-2, MCL-1, and BCL-xL, Drs. Kelly Goldsmith and Michael Hogarty facilitated a discussion on strategies to increase BCL-2 family protein inhibition efficacy and to reduce known toxicities, particularly with combination therapies; biomarker development to predict BCL-2 family protein inhibition efficacy; BCL-2 protein family inhibitor CNS penetration; and mechanisms of resistance to BCL-2 family protein inhibitors.

_Treatment Combinations to Maximize Efficacy_

Multiple lines of preclinical and clinical evidence indicate that BCL-2 inhibitors, when administered in combination with other targeted therapies, may augment their functions. Based on preclinical results, current adult clinical trials are assessing a number of therapies for administration with venetoclax, including: the BET inhibitor JQ-1, Notch inhibitors, aurora kinase inhibitors, immunotherapies, the mouse double minute 2 (MDM2) inhibitor idasanutlin, and navitoclax (BCL-2 and BCL-xL dual inhibitor). Efficacy and toxicology data from these adult clinical trials may inform the development of pediatric venetoclax combination regimens. Dr. Malcolm Smith noted disappointing results from clinical trials of BCL-2 family inhibitors plus chemotherapy in adult patients with solid tumors. Dr. Hogarty explained that mitochondrial function may affect BCL-2 family survival dependencies. For example, hematolymphoid tumors may be more sensitive to single agent inhibition than solid tumors due to more responsive mitochondria. Therefore, efficacy of BCL-2 family inhibitors could be improved with treatment strategies to amplify the pro-apoptotic, BCL-2-like 11 (BIM) load, which permits BAK/BAX oligomerization to overcome a threshold of attenuated mitochondrial apoptotic functions.

In addition, based on preclinical research of neuroblastomas, MCL-1 inhibitors likely need to be administered in combination with a BCL-xL inhibitor. While BCL-2 inhibition alone triggers a response in tumors dependent on BCL-2 for survival (i.e., BCL-2-dependent), tumors dependent on MCL-1 for survival (i.e., MCL-1-dependent) do not respond to single-agent MCL-1 inhibition. Dr. Hogarty explained that co-immunoprecipitation (co-IP) data from MCL-1-dependent neuroblastomas before and after MCL-1 inhibition indicate that after treatment, BIM was released from MCL-1 and subsequently bound to BCL-xL in all MCL-1-dependent neuroblastomas tested except for one model where BIM was displaced to BCL-2.
Multiple meeting participants agreed with the need for preclinical screens for therapeutic combinations strategies that include a BCL-2 protein family inhibitor combined with chemotherapy and novel therapies. These screens require development of a wide array of pediatric PDX models with differing survival dependencies. Different combinations will likely have varied effects in different tumor types and subtypes, but this screen should focus especially on promising tumor subtypes of neuroblastomas, osteosarcomas, and RMSs with evidence of potential BCL-2 protein family inhibitor sensitivity.

**Strategies to Mitigate Toxicities**

Preclinical and clinical evidence indicates that BCL-2 protein family inhibitors trigger hematological toxicities, particularly in certain therapeutic combinations of agents known to cause similar hematological effects. Because venetoclax and idasanutlin can each cause thrombocytopenia alone, preclinical assessment of additive hematological toxicities and determination of optimal dosing is necessary to support pediatric clinical development of this combination. In addition, the current M13-833 clinical trial results show that pediatric patients with solid tumors require discontinuous dosing of venetoclax when combined with Cy-Topo to avoid additive hematological toxicities (AbbVie, 2023a; Goldsmith et al., 2020).

Dr. Hogarty noted that MCL-1 inhibitor development may be lagging behind the development of other BCL-2 family protein inhibitors due to concerns related to cardiac toxicity. The MCL-1 inhibitor S63845 binds mouse MCL-1 with a 10-fold lower affinity compared to human MCL-1. Therefore, the use of mouse models with human MCL-1 is likely required to further understand MCL-1 inhibitor-related cardiac toxicities.

Mitigation of these hematological and cardiac toxicities requires development of a diverse array of appropriate preclinical pediatric cancer models. These preclinical models can be used to optimize dosing schedules and to develop and test new compounds, including PROTACs and ADCs.

**Dosing Schedules**

**Discontinuous**

Multiple meeting participants agreed that due to venetoclax’s role as a potentiator of other agents’ activities, discontinuous dosing is a rational strategy to minimize potential overlapping toxicities, even for more targeted therapies that lack significant hematological toxicities alone. Based on preliminary clinical data in neuroblastoma, Dr. Goldsmith suggested that discontinuous dosing of venetoclax and chemotherapy begin with venetoclax, followed by chemotherapy. However, based on BIM load and dynamics, Dr. Hogarty suggested that discontinuous dosing begin with chemotherapy to bolster BIM load, followed by venetoclax to displace BIM from BCL-2. Preclinical mouse models are well-positioned to test different dosing schedules that maximize treatment efficacy while minimizing toxicities. Notably, patients with different tumor types may have varying sensitivities to toxicities triggered by venetoclax treatment combinations. For example, patients with leukemias appear to tolerate continuous dosing of venetoclax plus chemotherapy better than patients with solid tumors. Therefore,
dosing schedules should be optimized for each tumor type to account for these differing sensitivities to toxicities.

**Metronomic Combination Therapies**

Dr. Goldsmith proposed testing metronomic treatment regimens involving non-tumor killing doses of BCL-2 protein family inhibitors in combination with other therapies that target the tumor microenvironment (TME) to increase tumor cell sensitivity to BCL-2 protein family inhibitors. Overall, this would enable lower dosing of BCL-2 protein family inhibitors and likely reduce severe toxicities.

**Development of New Compounds**

Preclinically, navitoclax demonstrated the highest efficacy in leukemias compared to other BCL-2 protein family inhibitors. However, thrombocytopenia may limit effective dosing in clinical trials. Therefore, alternatives to traditional small molecule inhibitors, such as PROTACs and antibody drug conjugates (ADCs), may provide more targeted therapeutics that do not affect platelets.

**Proteolysis Targeting Chimeras**

Early data suggest that a BCL-xL PROTAC degrader has less severe effects on platelets compared to other BCL-xL inhibitors because platelets may not contain the E3 ubiquitin ligase required for degradation. However, preclinical data on this BCL-xL PROTAC degrader is currently sparse and requires further preclinical testing.

**Antibody Drug Conjugates**

Dr. Smith suggested that incorporating a BCL-xL inhibitor as a payload in an ADC could also help to circumvent thrombocytopenia. Dr. Pooja Hingorani explained that AbbVie is currently developing two BCL-xL-conjugated ADCs with the following antibodies: B7 homolog 3 (B7H3) and epidermal growth factor receptor (EGFR). As monotherapies, neither of these ADCs are toxic and both are generally well tolerated. However, in clinical trials, co-administration of the B7H3-BCL-xL ADC with taxanes resulted in myolymphatic suppression, although the relative contribution of the ADC to this toxicity remains unclear. In addition, Dr. Goldsmith suggested that ADCs containing MCL-1 inhibitor moieties may result in fewer cardiac toxicities.

**Biomarker Development**

BCL-2 protein family survival dependencies are heterogenous within tumor types, and therefore robust biomarkers are required to administer BCL-2 protein family inhibitors to patients whose tumors are likely sensitive to these therapies. Moreover, accurate predictive biomarkers will eventually enable BCL-2 protein family inhibitors as first-line treatments by ensuring that mitochondrial function and sensitivity to these inhibitors remains intact and not attenuated as a result of initial therapies. Researchers can retrospectively assess potential RNA expression-based biomarkers using existing pediatric tumor RNA profiling data, but expression of BCL-2 protein family members is likely a poor surrogate for determining BCL-2 protein family survival dependencies. However, because MCL-1 is highly transcriptionally and post-
translationally regulated and has a very short half-life, high MCL-1 expression may indicate dependency.

Certain genetic alterations may also provide insight into tumor survival dependencies. Tumors with activating mutations in cytosolic mitogen-activated protein kinase (MAPK) signaling proteins (e.g., neurofibromatosis 1 [NF1], protein tyrosine phosphatase non-receptor type 11 [PTPN11], rat sarcoma viral oncogene [RAS], and rapidly accelerated fibrosarcoma [RAF]) are usually MCL-1-dependent, although these mutations are rare in pediatric cancers. Furthermore, MAPK signaling likely affects BCL-2 protein family survival dependence. For example, loss of NF1 in a BCL-2-dependent tumor results in BIM displacement to MCL-1, which is reversible with a MAPK inhibitor. However, the mechanism behind these effects remains unknown. MYCN amplified neuroblastomas are typically more sensitive to treatment combinations that include BCL-2 inhibition, but after BCL-2 inhibition, BIM likely switches binding to MCL-1, resulting in BCL-2 inhibitor resistance. However, Goldsmith explained that this particular sensitivity likely is unrelated to the MYCN amplifications and more specific to the other therapeutics being administered with these inhibitors; BCL-2 protein family inhibitors may be potentiating the effects of certain therapeutics being tested in MYCN amplified neuroblastomas.

Drs. Goldsmith and Hogarty agreed that protein-protein interaction assays are likely the most promising options for determining survival dependency. Importantly, because BCL-2 protein family survival dependencies across cells within a tumor are homogenous, bulk protein-protein interaction assays are sufficient for determining these dependencies. In preclinical settings, co-IP assays to assess BIM binding have successfully been used to identify BCL-2 protein family survival dependencies; tumors with high MCL-1-BIM binding are typically sensitive to MCL-1 plus BCL-xL inhibition, while tumors with high BCL-2-BIM are usually sensitive to BCL-2 inhibition. However, co-IPs require a lot of cellular material, which is infeasible for most solid tumors. Therefore, because it requires less tissue, researchers are trying to develop a proximity ligation assay using formalin fixed paraffin embedded (FFPE) tissue samples to enable clinical assessment of BIM binding.

Another strategy for biomarker development would be to correlate RNA expression profiles with tumor sensitivity to different BCL-2 protein family inhibitors. However, Dr. John Maris cautioned that while BCL-2 protein family dependencies may be relatively stable, cancer cells drastically change expression of different genes after different treatments and as they develop resistances.

Central Nervous System Penetration
The cerebrospinal fluid (CSF) to plasma ratio of a therapeutic is insufficient for assessing which inhibitors may be the most effective for CNS tumors. For example, the most effective tyrosine kinase inhibitors used to treat melanoma with CNS metastases do not always have the highest CSF to plasma ratios compared to other, less effective inhibitors.
Resistance Mechanisms
In vitro modeling has detected expected resistance mechanisms after treatment with a BCL-2 protein family inhibitor. As expected, treating a BCL-2-dependent neuroblastoma with a BCL-2 inhibitor triggers tumor cells to switch their survival dependency to MCL-1, rendering it resistant to BCL-2 inhibition. Rarely, exposing a BCL-2-dependent in vivo model to a BCL-2 inhibitor can trigger broad resistance to diverse classes of drugs, likely due to attenuated BAK and BAX signaling.

In addition, in vivo models exposed to a diverse set of tumor stressors that alter BAK and BAX signaling sometimes develop pan-resistance to BCL-2 protein family inhibitors, but the molecular mechanisms that enable the development of these resistances remain unknown. Therefore, previous treatment regimens and standard of care may already attenuate tumor sensitivities to BCL-2 protein family inhibitors administered as later line therapies in clinical trials of patients with r/r tumors. However, Dr. Goldsmith noted that in clinical practice, relapsed tumors still retain MCL-1 or BCL-2 dependence, even after exposure to different chemotherapies and immunotherapies.

Next Steps
BCL-2 family proteins are of moderate to high priority as targets for continued preclinical exploration. BCL-2 inhibitors are furthest along in preclinical development; thus, BCL-2 remains at a moderate priority, given that enough preclinical data already exists to support pediatric clinical trials of BCL-2 inhibitors for certain indications. However, MCL-1 and BCL-xL inhibitors likely require additional preclinical work to understand biological mechanisms triggered by these inhibitors, as well as to mitigate toxicities when administered in combination treatments. Therefore, MCL-1 and BCL-xL are of moderate-to-high priority drug targets.

Successful deployment of BCL-2 protein family therapies requires robust biomarkers to determine tumor survival dependencies and predict inhibitor efficacy. Moreover, developing biomarkers, as well as conducting preclinical efficacy studies of BCL-2 protein family inhibitors, requires more diverse tumor models with differing survival dependencies within each tumor type (e.g., a BCL-2-dependent neuroblastoma model and an MCL-1-dependent neuroblastoma model). In addition, because most tumor types require combination therapies with BCL-2 protein family inhibitors, preclinical drug combination screening can be leveraged to identify chemotherapies, targeted therapies, and novel drugs that are potentiated by BCL-2 family protein inhibitors. However, due to the risks of hematological and cardiac toxicities, especially when administered in combination therapies, BCL-2 family protein inhibitor preclinical studies should also focus on optimizing dosing schedules for each tumor type, as well as the development of more direct targeting strategies such as PROTACs and ADCs.
Appendix A: Preview of September Meeting Drug Targets

The next COACH meeting will occur on September 18, 2023, from 9:00 AM to 1:00 PM ET. During this meeting, participants will assess ADCs with three of the following antigens: CD19, CD22, CD33, CD123, GPC3, or a novel pediatric surface antigen. Dr. Maris requested that B7H3 targeted ADCs, as well as representation from companies with B7H3 targeted ADCs be included in this meeting to enable a more detailed discussion. Anyone with recommendations for ADC discussion leaders should contact FNIH.

The next round of target nomination and voting for the December 1, 2023 meeting will begin in July. FNIH will send an email requesting target nominations to organization leads.

Summaries of the third and fourth COACH meetings are nearing completion, and FNIH will share the finalized summaries with COACH members. In addition, FNIH will share infographics designed for lay audiences with COACH members, who are asked to then share these infographics on their social media platforms.
Appendix B: Bibliography


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Appendix B


City of Hope Medical Center. (2023). A Phase Ib Open Label Study of Navitoclax in Combination With Venetoclax + Decitabine in Relapsed/Refractory Acute Myeloid Leukemia Previously Treated With Venetoclax (Clinical Trial Registration No. NCT05222984). clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT05222984


Appendix B


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