Convening Experts in Oncology to Address Children’s Health

Quarterly Collaboration Meetings in Pediatric Oncology

December 1, 2023 | Virtual Meeting

Reviewed Targets:
- CCR8
- TEAD1
- HER2

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

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### Acronym Definitions

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<tbody>
<tr>
<td>AACR</td>
<td>American Association for Cancer Research</td>
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<tr>
<td>ACP5</td>
<td>acid phosphatase 5</td>
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<tr>
<td>ADCC</td>
<td>antibody-dependent cell-mediated cytotoxicity</td>
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<tr>
<td>ADC</td>
<td>antibody-drug conjugate</td>
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<td>AE</td>
<td>adverse event</td>
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<tr>
<td>Akt</td>
<td>AK strain transforming</td>
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<td>ALL</td>
<td>acute lymphoblastic leukemia</td>
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<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>BsAb</td>
<td>bispecific antibody</td>
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<tr>
<td>CAR-T</td>
<td>chimeric antigen receptor T cell</td>
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<tr>
<td>CCL1</td>
<td>chemokine ligand 1</td>
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<td>CD8</td>
<td>cluster of differentiation 8</td>
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<tr>
<td>CDK</td>
<td>cyclin-dependent kinase</td>
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<td>CDX</td>
<td>cell line-derived xenograft</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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<tr>
<td>COACH</td>
<td>Convening Experts in Oncology to Address Children’s Health</td>
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<td>COG</td>
<td>Children's Oncology Group</td>
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<tr>
<td>CPI</td>
<td>checkpoint inhibitor</td>
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<td>ctDNA</td>
<td>circulating tumor DNA</td>
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<tr>
<td>CTGF</td>
<td>connective tissue growth factor</td>
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<tr>
<td>Cyr61</td>
<td>Cysteine-rich angiogenic inducer 61</td>
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<tr>
<td>DSRCT</td>
<td>desmoplastic small round cell tumors</td>
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<tr>
<td>EAE</td>
<td>experimental autoimmune encephalomyelitis</td>
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<tr>
<td>EFS</td>
<td>event-free survival</td>
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<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
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<tr>
<td>EPHA2</td>
<td>ephrin type-A receptor 2</td>
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<td>EwS</td>
<td>Ewing sarcoma</td>
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<tr>
<td>Fc</td>
<td>fragment crystallizable</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>FLI1</td>
<td>Ewing sarcoma protein-friend leukemia integration 1</td>
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<td>FSG</td>
<td>focal segmental glomerulosclerosis</td>
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<td>GBM</td>
<td>glioblastoma multiforme</td>
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<td>GI</td>
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<td>GPC3</td>
<td>glypican 3</td>
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<tr>
<td>GPCR</td>
<td>G protein-coupled receptor</td>
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<td>HER2</td>
<td>human epidermal growth factor receptor 2</td>
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<tr>
<td>HL</td>
<td>Hodgkin’s lymphoma</td>
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<td>I-O</td>
<td>Immuno-oncology</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>IHC</td>
<td>immunohistochemistry</td>
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<tr>
<td>IL3Rα2</td>
<td>interleukin 13 receptor alpha 2</td>
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<tr>
<td>KO</td>
<td>knockout</td>
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<tr>
<td>LATS</td>
<td>large tumor suppressor kinase</td>
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<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
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<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
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<tr>
<td>MEK</td>
<td>mitogen-activated protein kinase kinase</td>
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<tr>
<td>MOA</td>
<td>mechanisms of action</td>
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<tr>
<td>MPNST</td>
<td>malignant peripheral nerve sheath tumor</td>
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<tr>
<td>MRD</td>
<td>minimal residual disease</td>
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<tr>
<td>NF2</td>
<td>neurofibromatosis type 2</td>
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<tr>
<td>nHL</td>
<td>non-Hodgkin’s lymphoma</td>
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<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
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<td>NSCLC</td>
<td>non-small cell lung cancer</td>
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<td>PD-1</td>
<td>programmed cell death protein 1</td>
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<td>PD-L1</td>
<td>programmed cell death ligand 1</td>
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<tr>
<td>PDX</td>
<td>patient-derived xenograft</td>
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<tr>
<td>PI3K</td>
<td>phosphoinositol 3-kinase</td>
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<tr>
<td>PIVOT</td>
<td>Pediatric Preclinical In Vivo Testing</td>
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<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
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<tr>
<td>PRAS40</td>
<td>proline-rich Akt substrate of 40 kilodaltons</td>
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<tr>
<td>PRISM</td>
<td>Profiling Relative Inhibition Simultaneously in Mixtures</td>
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<tr>
<td>r/r</td>
<td>relapsed/refractory</td>
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<tr>
<td>SHP2</td>
<td>Src homology 2 containing protein tyrosine phosphatase 2</td>
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<tr>
<td>SSM</td>
<td>simple somatic mutation</td>
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<tr>
<td>SV</td>
<td>structural variation</td>
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<tr>
<td>T-DXd</td>
<td>trastuzumab deruxtecan</td>
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<tr>
<td>TAZ</td>
<td>Tafazzin</td>
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<tr>
<td>TEAD1</td>
<td>TEA domain transcription factor 1</td>
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<tr>
<td>TME</td>
<td>tumor microenvironment</td>
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<tr>
<td>TOP1</td>
<td>topoisomerase 1</td>
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<tr>
<td>Treg</td>
<td>regulatory T cell</td>
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<tr>
<td>YAP</td>
<td>yes-associated protein</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 December 1, 2023, COACH convened the Seventh 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: chemokine receptor 8 (CCR8), TEA domain transcription factor 1 (TEAD1), and human epidermal growth factor receptor 2 (HER2).

**CCR8**

CCR8 is a cell surface receptor that belongs to class A of the G protein-coupled receptor (GPCR) family. CCR8 is associated with immunosuppressive functions of regulatory T cells (Tregs) that promote immune tolerance to tumor cells within the tumor microenvironment (TME), making CCR8 an attractive target for Treg depletion. Alterations in CCR8 are generally detected at a low frequency in pediatric cancers and not considered a major driver of carcinogenesis. Moreover, because CCR8 is primarily expressed on Tregs, RNA expression levels in bulk tumor tissue are uniformly low. Because CCR8 acts via Treg-mediated immunosuppression, cell-autonomous deletion of the gene has limited impacts on viability; all pediatric cell lines, including ALL and HL, show minimal CCR8 dependency in vitro. However, few studies have investigated the in vivo impact of CCR8 on tumor responses in key pediatric oncology indications, in part due to the lack of immune-competent preclinical mouse models. In addition, CCR8-targeting therapies have not entered pediatric clinical trials.

Upon initial characterization, Ccr8 knockout (KO) mice exhibited normal development, lifespan, and fertility, with no significant differences compared to wildtype mice in the development of lymphoid and hematopoietic organs. However, one study using an experimental autoimmune encephalomyelitis (EAE) mouse model with adoptive transfer of Ccr8 KO Tregs showed the importance of CCR8 expression in restraining EAE. In murine tumor models, anti-CCR8 antibodies have potent and specific effects on tumor-infiltrating Tregs due to their enriched expression of CCR8 compared to resident spleen or lymph node Tregs. Notably, Coherus’ anti-CCR8 monoclonal antibody (mAb) resulted in robust depletion of tumor-infiltrating Tregs, while sparing Tregs in healthy tissues.
Key Considerations

- Data confirming CCR8 expression in Tregs of pediatric solid tumors are needed to support a CCR8-targeted Treg depletion approach in pediatric indications.
- Existing and newly generated data on CCR8 expression in tumor-infiltrating Tregs need to be compared to data on CCR8 expression levels in circulating Tregs to confirm the selectivity of CCR8 expression within tumor-infiltrating Tregs.
- CCR8-targeting agents are effective in preclinical models in combination with standard care treatment, chemotherapy, and immuno-oncology (I-O) therapies.
- CCR8 inhibitors are effective in preclinical models in tumors that are resistant to PD-1 and PD-L1 inhibitors; however, whether Tregs are present in these tumors remains unclear.
- Treg depletion may cause autoimmune toxicities in pediatric patients, and the proportions of Treg subtypes change between childhood and adulthood.

Next Steps

- CCR8 is a low-to-moderate priority target for COACH.
- If adult clinical trials of CCR8-targeting therapies yield positive outcomes, appropriate pediatric preclinical models should be developed to evaluate these agents and identify the role of Tregs in pediatric tumor indications.

TEAD1

TEAD transcription factors contain a TEA domain that binds DNA elements and a transactivation domain that interacts with transcription co-activators such as yes-associated protein (YAP) and tafazzin (TAZ). Importantly, TEADs are key downstream transcription factors of the Hippo signaling pathway, which is often altered in human cancers, leading to the expression of pro-proliferation and anti-apoptosis genes. Multiple tumor types have shown overexpression of TEADs, especially TEAD1 and TEAD4 in some models, as well as YAP and TAZ, and TEAD expression levels correlate with poor clinical outcomes. Alteration rates for TEAD1 are generally low in pediatric cancers and not considered a major driver of carcinogenesis. However, tumor sensitivity to TEAD inhibition, dependent on Hippo pathway mutations, has been reported in mesothelioma, rare sarcomas, and ependymomas. TEAD1 mRNA is highly expressed in multiple solid tumor types, with specific enrichment in ependymomas. However, in vitro tumor expression of TEAD1 varies, with enrichment observed in central nervous system (CNS) tumors and sarcoma cell lines and gliomas, malignant peripheral nerve sheath tumors (MPNSTs), and osteosarcomas showing moderate sensitivity to loss of TEAD1. Importantly, several in vitro studies of TEAD1 drug inhibition have provided a rationale for interest in studying TEAD inhibition in cancers with Hippo pathway alterations.

Verteporfin, a YAP-TEAD inhibitor, has shown moderate activity in preclinical glioma and osteosarcoma models, as well as prolonged survival and reduced tumor size in a glioblastoma multiforme (GBM) G-13063 orthotopic patient-derived xenograft (PDX) model. Alongside TEAD1, TEAD2 inhibition also has demonstrated preclinical efficacy in tumor inhibition; blockade of YAP and TEAD via expression of a dominant-negative form of TEAD2 suppressed
tumor growth and prolonged survival in a hepatoblastoma mouse model. However, initial characterization of a Tead1 KO mouse model found that embryos died between embryonic days 11 and 12, exhibiting enlarged pericardial cavities, bradycardia, and dilated fourth brain ventricles. Conditional deletion of Tead1 in adult mouse cardiomyocytes is also lethal due to acute-onset dilated cardiomyopathy, and cardiac-specific Tead1 deletion phenocopied global loss of Tead1, highlighting an essential role for Tead1 in cardiac homeostasis. In addition, early adult clinical trials of YAP-TEAD inhibitors reported several on-target kidney-related toxicities, including proteinuria and albuminuria. TEAD1-targeted therapies have not entered pediatric clinical trials.

Key Considerations

- Preclinical studies of Tead KO mice and adult clinical trials of TEAD inhibitors have shown that TEAD inhibition can introduce on-target renal toxicity, although it remains unclear which TEAD protein targets are responsible for this toxicity.
- Verteporfin’s activity is non-selective to specific TEADs and introduces off-target effects in studies of YAP-TEAD function.
- Tight temporal control of agent administration may also be required for optimal efficacy against persister cells and mitigation of potential renal toxicities.
- Many tumors contain drug-tolerant persister cells that are dependent on YAP/TAZ signaling for survival; thus, a multi-agent strategy may be necessary to prevent YAP/TAZ signaling from inducing tumor survival and potential relapse.
- Because minimal residual disease (MRD) is correlated with persister cell survival, clinical trials should consider monitoring circulating tumor DNA (ctDNA) in patients’ blood.

Next Steps

- TEAD1 is a moderate-to-high priority target for COACH.
- Continued monitoring of current adult clinical trials will provide insights into safety profiles and TEAD subtype-specific functions.
- Implementing delayed administration of broad TEAD inhibitors and subtype-specific inhibitors in preclinical studies could provide evidence for shortening administration timeframes to reduce potential toxicities.

HER2

HER family proteins are type I transmembrane growth factor receptors that activate key intracellular signaling pathways. HER receptors play essential roles in the development and maintenance of mammary, cardiac, and neural tissues. Increased HER activation leads to activation of its two downstream pathways—mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/Akt signaling—resulting in increased proliferation and decreased cell death. Alterations in HER2 are rare in pediatric indications and not major drivers of pathogenesis; however, HER2 mRNA is expressed in multiple pediatric solid tumors with enrichment in ependymomas, rhabdoid tumors, and Wilms tumors, and desmoplastic small round cell tumors (DSRCT). Many pediatric cancer cell lines are moderately sensitive to HER2 loss in vitro, with solid tumor indications showing greater dependency than hematologic and
lymphatic cancers. In addition, a variety of HER2-targeting drug modalities have shown in vitro activity. In vivo, HER2 targeting has shown potential as a therapeutic target for some solid tumors, which is supported by strong preclinical efficacy in multiple indications. Specifically, trastuzumab deruxtecan (T-DXd), a HER2-targeting ADC, has demonstrated a potential as an effective therapeutic modality in pediatric solid tumor models, including Wilms tumor, rhabdoid tumor, and osteosarcoma. In addition, HER2 monovalent and trivalent CAR-T therapies have shown efficacy in multiple ependymoma and medulloblastoma models and demonstrated significant tumor reduction and survival benefits in multiple xenograft ependymoma models.

HER2 gene disruption and drug targeting is associated with significant risk of cardiotoxicity, which can be mitigated with cardioprotective agents or novel drug modalities. Her2 KO mice exhibit severe defects in the development of sensory and motor neuronal systems and die early in embryonic development due to dysfunctions associated with a lack of cardiac trabeculae. For Herceptin (Genentech-produced trastuzumab), significant cardiac toxicity has been reported in adult patients, particularly in anthracycline-based trastuzumab regimens. However, a phase II trial of trastuzumab and anthracycline-based chemotherapy in pediatric osteosarcoma patients found no evidence of acute cardiotoxicity when patients received the cardioprotective agent dexrazoxane. The HER2 inhibitor lapatinib was well tolerated, but showed little single agent activity, in past trials in pediatric CNS malignancies. More recent phase I and II trials have found varying efficacy of HER2-targeting ADCs and CAR-T cell therapies, primarily in patients with HER2-positive tumors. There are 34 HER2-targeted products approved worldwide, creating opportunities for clinical trials to provide rapid insight into the efficacy of these products in pediatric indications.

Key Considerations

- The pediatric oncology community has shown low interest in developing agents that inhibit HER2 kinase activity due to the lack of genomic alterations in pediatric cancers that lead to activation of HER2.
- HER2-targeting ADCs have shown anti-tumor activity in preclinical studies of Wilms and rhabdoid tumors DSRCT, which warrants further testing in pediatric clinical trials.
- It is not clear whether the effectiveness of T-DXd is due to successful HER2 targeting for payload delivery or a bystander effect (i.e., systemic, non-targeted exposure to the payload).
- HER2 IHC findings are difficult to interpret, especially in low-expressing HER2 histologies, due to variability in antibodies used to detect HER2 via IHC.
- For future therapies, researchers should consider HER2 kinase activity inhibition, HER2-targeting ADCs using novel payloads and linkers, and HER2-targeting CAR-T cell therapies for agent development.

Next Steps

- HER2 is a low-to-moderate priority target for COACH.
- Additional preclinical studies should provide insights into MOAs for other HER2-targeting agents and methods to enhance HER2 detection using IHC.
• A basket clinical trial studying the efficacy of T-DXd in treating tumors with high HER2 expression can provide rapid insights into the relevance of HER2 antibodies for payload delivery by HER2-targeting ADCs.
• A clinical trial evaluating Wilms tumor and rhabdoid tumors warrants consideration. Moreover, given promising preclinical activity in HER2 low/zero models, clinical investigation in HER2 low/zero tumors should also be considered.
• Additional preclinical studies are needed to better understand the preclinical activity observed in HER2 low/zero pediatric tumors and provide insights into potential alternative response biomarkers.
Meeting Summary

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

Dr. Stacey Adam welcomed meeting participants and reaffirmed the goal of Convening Experts in Oncology to Address Children’s Health (COACH)—to provide expert recommendations regarding drug target prioritization and salient preclinical steps to either clarify the priority of a specific drug target, or prepare a drug target, for pediatric cancer clinical trials. For targets identified as high priority, meeting participants 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. For targets identified as low priority, participants will determine whether additional preclinical data could advance the target to a higher priority or declare it as not relevant for pediatric cancer indications. 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, and 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, FNIH

Dr. Adam presented data on relevant genetic alterations, transcriptomic expression, patient survival, and in vitro and in vivo dependency for three drug targets—chemokine receptor 8 (CCR8), TEA domain transcription factor 1 (TEAD1), and human epidermal growth factor receptor 2 (HER2)—as well as data on in vitro and in vivo drug sensitivity and clinical response rates for their respective therapeutics. Within a given tumor type, the presence of alterations in a drug target does not necessarily indicate its suitability as an effective therapeutic target. Similarly, a therapeutic formulated to inhibit a differentially expressed protein in a specific tumor type may not result in a 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 consider these data in combination, identifying specific pediatric indications that may be sensitive to target therapeutics.

Data types for each drug target were compiled using different databases, as well as scientific literature. Data on relevant genetic alterations, compiled from cBioPortal and PedcBioPortal cohorts, included simple somatic mutations (SSMs), copy number variations (CNVs), and structural variations (SVs); and patient expression data were compiled from CCDI Molecular Targets Platform and XenaBrowser. In vitro dependency data, obtained from Dependency Map (DepMap), were presented using Chronos scores, a normalized metric of cell viability after gene deletion, with a Chronos score of 0 indicating that a gene is non-essential, while a score of -1
indicates comparable dependency to the median of all pan-essential genes. DepMap data on *in vitro* sensitivity were represented using Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) scores, with area under the curve (AUC) derived from an 8-point dose-response curve ranging from 10µM to 610pM; a PRISM score of 1 indicates complete lack of response at all concentrations, whereas a 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.

**CCR8**  
*Stacey Adam, PhD, FNIH*

**Overview**  
CCR8 is a cell surface receptor that belongs to class A of the G protein-coupled receptor (GPCR) family. It has four known ligands, of which the best characterized is chemokine ligand 1 (CCL1) (Schaerli et al., 2004). CCR8 is associated with immunosuppressive functions of regulatory T cells (Tregs), which promote immune tolerance to tumor cells within the tumor microenvironment (TME) (Ohue & Nishikawa, 2019). Enriched CCR8 expression in tumor-resident Tregs was first identified in breast cancer and has been documented in multiple cancers (Plitas et al., 2016). In healthy tissues, CCR8-expressing T cells are primarily found in the skin and rarely in peripheral blood. CCR8 is an attractive target for Treg depletion because its low expression in normal healthy tissues enables specific anti-tumor activity of effector T cells. However, systemic depletion of Tregs can lead to severe autoimmune toxicities (Glasner & Plitas, 2021). Other potential Treg targets such as CD25 and CCR4 showed broad expression on peripheral Tregs (Ohue & Nishikawa, 2019).

**Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival**  
Alterations in CCR8 are generally detected at a low frequency in pediatric cancers and not considered a major driver of carcinogenesis. Because CCR8 is primarily expressed on Tregs, mRNA expression levels in bulk tumor tissue are uniformly low. In addition, the CCR8 ligand chemokine ligand 18 (CCL18), expressed by immune cells in glioma models, may be of therapeutic relevance (W. Gao et al., 2022). CCR8 expression is not associated with survival across key pediatric oncology indications, although CCR8 expression has been associated with poor prognosis in adult non-small cell lung cancer (NSCLC) and colorectal cancers (De Simone et al., 2016).
Dependency and Drug Sensitivity

**In Vitro Dependency and Sensitivity**
Expression of CCR8 in pediatric cancer cell lines is highly restricted to lymphatic cancers, specifically to acute lymphoblastic leukemia (ALL) and Hodgkin’s lymphoma (HL). Studies have also reported high expression in adult T cell lymphomas and leukemias, as well as cutaneous T cell lymphomas (Giustiniani et al., 2022; Zheng et al., 2022). Conversely, in solid tumor cell lines, CCR8 is primarily expressed on immune cells in the TME, and not by cancer cells themselves.

Because CCR8 acts via Treg-mediated immunosuppression, cell-autonomous deletion of the gene has limited impacts on viability; all pediatric cell lines, including ALL and HL, show minimal CCR8 dependency *in vitro*. However, activation of CCR8 has been shown to promote glioma progression via the acid phosphatase 5 (ACP5)/ proline-rich Akt substrate of 40 kDa (PRAS40)/AK strain transforming (Akt) signaling cascade *in vitro*. In addition, knockdown of CCR8 in U251MG glioma cells attenuated the pro-tumorigenic effects of CCL18 expressed by humanized macrophages, resulting in significantly smaller tumor volume (Huang et al., 2022).

**In Vivo Sensitivity**
Few studies have investigated the *in vivo* impact of CCR8 on tumor responses in key pediatric oncology indications, partly due to the lack of immune-competent preclinical mouse models. Thorough preclinical evaluation of CCR8 therapies requires use of syngeneic tumor models or humanized immune system models (Olson et al., 2018). Compared to patient-derived xenografts (PDXs) and human cell line xenografts, these models are limited and experimentally complex, in part due to variable phenotypes and a lack of a native TME.

CCR8 therapies have shown promise in combination with programmed cell death protein 1 (PD-1) inhibition in adult cancers, but low sensitivity to PD-1 inhibition in most pediatric tumors limits this approach. However, combination CCR8 and PD-1 inhibition resulted in synergistic anti-tumor efficacy in a mouse model resistant to PD-1 inhibition, MBT-2 (Weaver et al., 2022).

Recent preclinical data from American Association for Cancer Research (AACR) 2023 Annual Meeting highlight a growing interest in CCR8 as a therapeutic target for adult cancers:

- Anti-CCR8 IPG0521 inhibited growth of lung, liver, breast cancers in both syngeneic and immune-humanized mouse models by inhibiting Treg and activating cluster of differentiation 8 (CD8) positive T cells (Fan et al., 2023).
- Domain Therapeutics presented work on CCR8-directed monoclonal antibodies (mAbs) validated in human CCR8 knock-in mice (Richert et al., 2023; Vermot-Desroches et al., 2023).
- Coherus’ SRF114 asset showed single agent activity in checkpoint-resistant models, highlighting an immune checkpoint inhibitor (CPI)-independent role for Treg cell depletion (Panduro et al., 2023).
• Biocytogen developed a triple humanized PD-1/programmed cell death ligand 1 (PD-L1)/CCR8 mouse model to show \textit{in vivo} efficacy of anti-PD-1 and anti-CCR8 combination therapy (L. Wang et al., 2023).

\textbf{Preclinical Safety and Toxicology}

Upon initial characterization, \textit{Ccr8} knockout (KO) mice exhibited normal development, lifespan, and fertility, as well as no significant differences compared to wildtype mice in the development of lymphoid and hematopoietic organs (Chensue et al., 2001). Using models of Type 2 helper T cell (Th2)-mediated immune responses and Th2-mediated allergic airway disease, Chensue et al. showed impairment of Th2-type cytokine expression and eosinophil mobilization in \textit{Ccr8} KO mice. Notably, CCR8 deficiency had no effect on the Th1-type response to mycobacterial antigens, indicating the immune defects were Th2-specific.

CCR8-positive Tregs are key drivers of immunosuppression in normal immune responses (Barsheshet et al., 2017). In human peripheral blood cells, more than 30 percent of Tregs up-regulate CCR8 following ligand-dependent activation by CCL1. In an experimental autoimmune encephalomyelitis (EAE) mouse model with adoptive transfer of \textit{Ccr8} KO Tregs, Barsheshet et al. showed the importance of CCR8 expression in restraining EAE.

In murine tumor models, anti-CCR8 antibodies have potent and specific effects on tumor-infiltrating Tregs due to enriched expression of CCR8 in this population over resident spleen or lymph node Tregs (Panduro et al., 2023). Coherus’ anti-CCR8 mAb resulted in robust depletion of tumor-infiltrating Tregs, whilst sparing Tregs in healthy tissues.

\textbf{Clinical Trial Development}

CCR8-targeting therapies have not entered pediatric clinical trials.

\textbf{Research and Development Landscape}

Interest in CCR8 in literature has fluctuated greatly since its first publication in 1990, with a recent spike of interest in 2021 and 2022. Literature suggests low interest in CCR8 as potential target in oncology, given that only 25 percent of CCR8 articles are oncology related. Non-Hodgkin’s lymphoma (nHL), HL, and glioma are the only referenced FNIH indications in CCR8 literature.

There are currently no Food and Drug Administration (FDA)-approved CCR8-targeted products. The ten drugs that have progressed furthest in the pipeline are in Phase I and Phase II clinical trials and are primarily mAbs, and ten additional drugs are in preclinical development.

\textbf{Additional Considerations for Preclinical and Clinical Development}

\textit{Facilitated by Alexander Scholz, PhD, Gilead Sciences}

\textbf{CCR8 Expression Levels}

Data confirming the expression of CCR8 in Tregs of pediatric solid tumors are needed to support a CCR8-targeted Treg depletion approach in pediatric indications. Furthermore, bulk RNA sequencing (RNASeq) data from tumor samples may not help identify a population of tumor-
infiltrating Tregs with CCR8 expression because only a small fraction of cells within the TME will be positive. To confirm the expression of CCR8 in Tregs, researchers must evaluate immunohistochemistry (IHC) staining of CCR8 with CD4-positive tumor-resident Tregs using a flow cytometry approach that allows the isolation of CCR8-positive Tregs from primary tumor samples. The Pediatric Preclinical In Vivo Testing (PIVOT) Consortium can also query existing tissue microarray, single cell RNASeq, and single nucleus RNASeq data from serial sections of pre- and post-relapse patient clinical specimens to assess CCR8 expression in Tregs in both disease states. Overall, these data need to be compared to data on CCR8 expression levels in circulating Tregs to confirm the selectivity of CCR8 expression within tumor-infiltrating Tregs.

CCR8 is expressed in ALL and HL, which may support targeting CCR8 within primary tumors rather than depleting Tregs. However, expression levels of CCR8 may not be high enough to allow for CCR8-targeting agents to directly target primary tumors.

**Efficacy of CCR8-targeting/Treg-depleting Agents**

Most Treg-depleting agents are mAbs—designed to optimize mutations in the fragment crystallizable (Fc) antibody domain—which have shown significant anti-tumor activity. All clinical trials for CCR8-targeting therapies are in early phases with no available data. However, in preclinical studies using adult tumor models, CCR8-targeting agents are effective in combination with standard of care treatment, chemotherapy, and immuno-oncology (I-O) therapies. Moreover, CCR8 inhibitors are effective in tumors resistant to PD-1 and PD-L1 inhibitors; however, whether Tregs are present in these tumors remains unclear. Further preclinical research should focus on identifying pediatric tumor types in which Tregs are present and understanding the role of T cell immunity in these tumors.

**Potential Toxicities from Treg Depletion**

Depleting Tregs may cause autoimmune toxicities in pediatric patients. In addition, prior studies showed that the proportion of Tregs and other T cell subtypes changes from childhood to adulthood, necessitating further study of Treg depletion in immunocompetent pediatric preclinical models.

**Next Steps**

In summary, CCR8 is a low-to-moderate priority target—scoring 1.9 out of 5 in the online poll—based on the lack of data detailing CCR8 expression in Tregs. Before considering pediatric clinical trials for CCR8-targeting agents, researchers should analyze existing IHC, single cell RNASeq, and single nucleus RNASeq data to determine whether CCR8 expression in Tregs is high compared to other tissues in pediatric patients. If adult clinical trials of CCR8-targeting therapies yield positive outcomes, researchers should then identify the role of Tregs in pediatric tumor indications and develop appropriate pediatric preclinical models for evaluating these agents.
TEAD1
Stacey Adam, PhD, FNIH

Overview
TEAD transcription factors contain a TEA domain that binds DNA elements and a transactivation domain that interacts with transcriptional co-activators such as yes-associated protein (YAP) and tafazzin (TAZ) (Holden & Cunningham, 2018). TEADs are also key downstream transcription factors of the Hippo signaling pathway, which is often altered in human cancer, leading to expression of pro-proliferation and anti-apoptosis genes (Cunningham & Hansen, 2022).

Multiple tumor types have shown overexpression of TEADs, especially TEAD1 and TEAD4 in some models, as well as YAP and TAZ, and TEAD expression levels correlate with poor clinical outcomes (Holden & Cunningham, 2018). Furthermore, TEAD1 expression levels have been directly linked to the expression levels of pro-growth factors such as connective tissue growth factor (CTGF), cysteine-rich angiogenic inducer 61 (Cyr61), AXL, Myc and survivin. Overall, TEAD1 is an attractive therapeutic target due to both its role as a critical mediator of tumorigenesis and its high expression in pediatric indications.

Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival
Alteration rates of TEAD1 are generally low in pediatric cancers and not considered a major driver of carcinogenesis. However, sensitivity to TEAD inhibition that is dependent on Hippo pathway mutations has been reported in mesothelioma, rare sarcomas, and ependymomas. Neurofibromatosis type 2 (NF2) gene mutations are common in mesothelioma, making TEAD inhibition a promising therapeutic strategy in this cancer type (Bueno et al., 2016; Tang et al., 2021). In addition, YAP-TAZ gene fusions can drive tumorigenesis in rare sarcomas (Lamar et al., 2018), and YAP fusions with other transcription factors and co-activators have been reported in pediatric ependymomas and have been shown to initiate tumorigenesis in mouse models (Eder et al., 2020; Pajtler et al., 2019). Lastly, Ewing sarcoma protein (EwS)-friend leukemia integration 1 (FLI1) transcription factor fusions in Ewing sarcoma perturb YAP1/TEAD signaling (Katschnig et al., 2017).

TEAD1 mRNA is highly expressed in multiple solid tumor types, with specific enrichment in ependymomas, and alternative splicing of TEAD1 mRNA produces isoforms with various functions and oncogenic properties (Choi et al., 2022). One study reported an association of TEAD1 with survival in osteosarcoma patients; however, the significance is unclear (Bian et al., 2022). Tumors may also preferentially express other TEAD family members, such as TEAD4, which is enriched in hepatoblastomas (Zhang et al., 2019).

Dependency and Drug Sensitivity

In Vitro Dependency and Sensitivity
In vitro tumor expression of TEAD1 varies, with enrichment observed in central nervous system (CNS) tumors and sarcoma cell lines, and gliomas, malignant peripheral nerve sheath tumors (MPNSTs), and osteosarcomas showing moderate sensitivity to loss of TEAD1. In osteosarcoma
cells, silencing of TEAD1 suppressed malignant phenotypes, including cell proliferation, apoptosis resistance, and invasive potential (Chai et al., 2017). TEAD4 silencing also inhibits YAP target gene expression and promotes apoptosis in hepatoblastoma cell lines, underscoring the potential of TEAD inhibitors more broadly (Zhang et al., 2019). In addition, YAP/TAZ inhibition reduces metastatic potential of EwS cells in vitro (Bierbaumer et al., 2021).

Several in vitro studies of TEAD1 drug inhibition provide a rationale for additional research in cancers with Hippo pathway alterations:

- IK-930 inhibited proliferation of Hippo pathway-deficient cancer cell lines, but not wildtype cells (Amidon et al., 2022).
- The broad YAP-TEAD inhibitor verteporfin impaired in vitro migration dynamics in primary and recurrent glioblastoma multiforme (GBM) lines (Barrette et al., 2021).
- VT3989 showed synergistic inhibition of proliferation with osimertinib in several EGFR mutant NSCLC cell lines (Tang & Post, 2022).

**In Vivo Sensitivity**
Verteporfin has shown moderate activity in preclinical glioma and osteosarcoma models, and prolonged survival and reduced tumor size in a GBM G-13063 orthotopic PDX model (Barrette et al., 2021). Although single agent verteporfin had limited activity in a recurrent GBM G-16302 PDX model, combination with temozolomide and radiotherapy significantly prolonged survival. In addition, verteporfin inhibits growth of an osteosarcoma Saos-2 xenograft, downregulating downstream target genes of TEAD1 (Yang et al., 2021). Although studies on verteporfin have provided evidence of the survival dependency of TEADs in multiple cancer indications, due to its mechanism of action (MOA) as a photosensitizer, verteporfin’s activity is non-selective to specific TEADs and introduces off-target effects in studies of YAP-TEAD function.

Alongside TEAD1, TEAD2 inhibition also has demonstrated preclinical efficacy in tumor inhibition. Blockade of YAP and TEAD via expression of a dominant-negative form of TEAD2 suppressed tumor growth and prolonged survival in a hepatoblastoma mouse model. In addition, increased YAP1 expression or deletion of YAP1 regulators—large tumor suppressor kinase [LATS] 1/2—drive ependymoma-like tumor formation in mice (Eder et al., 2020).

Although TEAD inhibition in preclinical pediatric tumor models has not been extensively studied, AACR 2023 Annual Meeting showcased many late-stage preclinical candidates that target TEADs:

- BPI-460372, a novel small molecule inhibitor of TEAD auto-palmitoylation, inhibits proliferation of tumor cells harboring Hippo pathway aberrations and suppresses growth in NF2-deficient or LATS1/2 mutant xenograft models (Shen et al., 2023).
- Hanmi Pharmaceutical presented data on oral TEAD inhibitors, which suppressed tumor growth at tolerable doses in xenograft mice harboring NF2 alterations (Kim et al., 2023).
- Ikena Oncology’s IK-930 is a paralog-specific TEAD inhibitor that showed an improved safety profile over pan-TEAD inhibitors (Young et al., 2023).
• TY-0584 showed efficacy in mesothelioma H226 cell line-derived xenograft (CDX) models, as well as head and neck cancer PDX models (Liang et al., 2023).
• BGI-9004, a covalent inhibitor of TEAD1-4, induced sustained tumor regression in NF2-deficient NCI-H226 and LATS-mutant MST-O211H mesothelioma xenograft models (Guo et al., 2023).

Preclinical Safety and Toxicology
Initial characterization of a Tead1 KO mouse model found that embryos died between embryonic days 11 and 12, exhibiting enlarged pericardial cavities, bradycardia, dilated fourth brain ventricles (Chen et al., 1994). Few studies have examined the effects of Tead1 loss in adult mice (Kakiuchi-Kiyota et al., 2019), but recent studies have started to elucidate tissue-specific roles for Tead1 in post-embryonic development. Conditional deletion of Tead1 in adult mouse cardiomyocytes is lethal due to acute-onset dilated cardiomyopathy, while cardiac-specific Tead1 deletion phenocopied global loss of Tead1, highlighting an essential role of Tead1 in cardiac homeostasis (J. Liu et al., 2021; R. Liu et al., 2017). Mechanistically, loss of Tead1 induces necroptosis via increased mitochondrial reactive oxygen species, disrupted mitochondrial structure, and reduced complex I-IV driven oxygen consumption. A first-in-human (FIH) trial of a YAP-TEAD inhibitor reported one case of Grade 4 cardiomyopathy possibly related to treatment (Yap et al., 2023).

Genetic targeting of YAP leads to focal segmental glomerulosclerosis (FSG) and progressive renal failure in mice (Schwartzman et al., 2016). In addition, early adult clinical trials of YAP-TEAD inhibitors reported several kidney-related toxicities including proteinuria and albuminuria (Yap et al., 2023). However, novel agents with paralog-specific actions have shown limited kidney toxicity in rats above efficacious doses and no signs of renal toxicities in non-human primates (Young et al., 2023).

Clinical Trial Development
TEAD1-targeted therapies have not entered pediatric clinical trials.

Research and Development Landscape
Publications focused on TEAD1 have fluctuated, with the volume of articles generally increasing since 2014 and peaking in 2021. Preclinical investigations into TEAD1 as an oncology target started in 2016, but the number of publications each year has been inconsistent. Currently, glioma is the most mentioned indication in TEAD1 articles, with 6 references.

There are no FDA-approved TEAD1-targeted products; the four assets (BPI-460372, IAG933, IK-930 and VT3989) that have progressed furthest in the pipeline are in Phase I clinical trials.
Additional Considerations for Preclinical and Clinical Development

Facilitated by Tracy Tang, PhD, Vivace Therapeutics, and John Maris, MD, Children’s Hospital of Philadelphia

Potential Pediatric Renal Toxicity

Preclinical studies of Tead KO mice and adult clinical trials of TEAD inhibitors have shown that TEAD inhibition can introduce on-target renal toxicity, although it remains unclear which TEAD protein targets are responsible for this toxicity. The safety profile of TEAD inhibitors is further complicated by mutant isoforms of TEAD proteins produced by alternative splicing of the gene that provide tumor suppressive effects, which TEAD inhibitors can reduce. Additional preclinical models are needed to understand the functions of specific TEADs.

Adult clinical trials have monitored renal protein levels (e.g., urea and albumin) and have found that any abnormal levels are resolved once TEAD inhibitor administration is discontinued. However, any effects of Hippo pathway inhibition on organ development in children and young adults could be permanent. Additional preclinical studies are needed to understand the role of TEAD in kidney development.

Persister Cell Adaptation

Many tumors contain drug-tolerant persister cells dependent on YAP/TAZ signaling for survival. Normally, the Hippo pathway is regulated by cell density. When cell density is low, pathway signaling is suppressed, increasing the expression of YAP/TAZ target genes and inducing anti-apoptotic and proliferative functions; this reduced apoptosis and increased proliferation eventually increases cell density, in turn reactivating the Hippo pathway. Thus, a multi-agent strategy may be necessary to prevent YAP/TAZ signaling from inducing tumor survival/persistence and potential relapse. Pan-TEAD inhibitors that broadly block all TEAD proteins may also be necessary to inhibit compensation for loss of TEAD1 by other TEAD proteins; however, this strategy may increase potential toxicities.

Typically, multiple agents are administered at suboptimal doses simultaneously to induce a significant combinatorial therapeutic effect. However, tight temporal control of agent administration may be required to optimize efficacy against persister cells and mitigate potential renal toxicities. Agents such as mitogen-activated protein kinase (MEK) and epidermal growth factor receptor (EGFR) inhibitors can be administered to initially debulk tumors, with TEAD inhibitors administered subsequently to eliminate persister cells. Several studies presented at the AACR Annual Meeting found that either MEK or EGFR inhibitors in combination with TEAD inhibitors delayed tumor relapse in PDX models, compared to MEK or EGFR inhibitors alone (i.e., single agent treatment). However, additional preclinical studies are needed to understand whether the delayed, short-term administration of a TEAD inhibitor can effectively reduce persister cell YAP/TAZ signaling and increase event-free survival (EFS).

Because minimal residual disease (MRD) is correlated with persister cell survival, clinical trials should consider monitoring circulating tumor DNA (ctDNA) in patients’ blood. By measuring ctDNA, TEAD inhibitor administration may be able to be restricted to only when ctDNA is
detected, which can reduce potential renal toxicity. However, studies have found that some
neuroblastomas do not shed ctDNA despite tumor burden, although this was often observed in
older patients. Implementing a combination of mutation-specific gene panels, low-pass whole
genome sequencing, and C-Circle assays in clinical trials may enhance ctDNA detection
sensitivity.

Next Steps
In summary, TEAD1 is a moderate-to-high priority target—scoring 3.4 out of 5 in the online
poll—based on TEAD1’s expression profile, the ability of TEAD inhibitors to eliminate persister
cells, and the current success of combination therapies. To better understand safety profiles
and TEAD subtype-specific functions, researchers should continue monitoring adult clinical
trials and evaluating delayed administration of broad TEAD inhibitors and subtype-specific
inhibitors in preclinical studies. Experts in kidney development and renal function from the
International Pediatric Renal Tumor Biology meeting can potentially provide insights into
adverse effects from disrupting the Hippo pathway in children.

HER2
Stacey Adam, PhD, FNIH

Overview
HER family proteins are type I transmembrane growth factor receptors that activate key
intracellular signaling pathways (Moasser, 2007). HER receptors play essential roles in the
development and maintenance of mammary, cardiac, and neural tissues. Gene disruption
models in mice demonstrate HER receptors’ critical role in the development of multiple organ
systems including brain, skin, lung, and gastrointestinal (GI) organs. Downstream pathways
include mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/Akt
signaling (Edwards et al., 2006).

Increased HER activation leads to activation of its downstream pathways, resulting in increased
proliferation and decreased cell death. Aberrant activation of HER can occur due to receptor
overexpression, mutational activation, or in response to elevated levels of growth factors.
Within the HER family proteins, HER2 possesses the strongest catalytic activity, making it an
attractive therapeutic oncology target.

Relevant Genetic Alterations, Transcriptomic Expression, and Patient Survival
Alterations in HER2 are rare in pediatric indications and are not considered major drivers of
pathogenesis. Studies of pediatric osteosarcomas have not detected HER2 amplification or
overexpression (Somers et al., 2005), and a Children's Oncology Group (COG) study of pediatric
osteosarcoma found no prognostic associations with HER2 expression (Gorlick et al., 2014).

Despite HER2 alterations not being a significant driver of pediatric cancers, HER2 mRNA is
expressed in multiple pediatric solid tumors, with enrichment in ependymomas, rhabdoid
tumors, and Wilms tumors. Wilms tumor RNA expression has been validated by multiple
studies, and PDX models showed high RNA expression in ependymomas (Hingorani et al., 2022;
Pinthus et al., 2004; Ragab et al., 2010; Salem et al., 2006). However, HER2 expression in retinoblastoma may be a truncated protein lacking the trastuzumab binding site (Seigel et al., 2016).

**Dependency and Drug Sensitivity**

**In Vitro Dependency and Sensitivity**
Most pediatric cancer cell lines show relatively low HER2 expression compared to adult cancers, although breast and GI tract cancers have significant proportions of HER2 high-expressing tumors. Despite typically low expression, many pediatric cancer cells are moderately sensitive to HER2 loss in vitro, with solid tumor indications showing greater dependency than hematologic and lymphatic cancers.

A variety of HER2-targeting drug modalities have shown in vitro activity. The HER2 inhibitor lapatinib inhibits proliferation, increases apoptosis, and impairs cell migration of U2-OS and MG-63 cells (Long et al., 2014). In addition, HER2-targeted chimeric antigen receptor T cell (CAR-T) therapy eliminates tumor-initiating cells in osteosarcoma in vitro models (Rainusso et al., 2012).

**In Vivo Sensitivity**
HER2 targeting has shown potential as a therapeutic target for solid tumors, which is supported by strong preclinical efficacy in multiple indications. Trastuzumab deruxtecan (T-DXd), a HER2-targeting ADC, has demonstrated therapeutic efficacy in pediatric solid tumor models (Hingorani et al., 2022). T-DXd induced prolonged EFS in pediatric PDX models of Wilms tumor and rhabdoid tumors. T-DXd also showed modest anti-tumor activity in breast cancer patients with a HER2 IHC score of 0, suggesting that anti-tumor activity observed in preclinical models with low HER2 expression may have clinical relevance (Modi et al., 2022; Mosele et al., 2023).

Anti-tumor activity of other HER2-targeting therapies has been demonstrated in osteosarcoma. A cisplatin-loaded HER2 affibody (i.e., antibody mimetic)-based conjugate demonstrated selective tumor uptake and efficacy in inducing apoptosis and ferroptosis, as well as the ability to overcome drug resistance from cisplatin resistant cells by inducing ferroptosis in vitro (He et al., 2023). In addition, HER2-bispecific antibody (BsAb)-armed T cell therapy has shown significant anti-tumor activity in multiple osteosarcoma PDX models (143B, OSOS1B, TESOSC1, and HGSOS) without significant toxicity (Park & Cheung, 2020); combination with atezolizumab further enhanced tumor reduction in the 143B xenograft model.

HER2 monovalent and trivalent (HER2, interleukin 13 receptor alpha 2 [IL13Rα2], and ephrin type-A receptor 2 [EPHA2]) CAR-T therapies have shown efficacy in multiple ependymoma and medulloblastoma models and demonstrated significant tumor reduction and survival benefits in MDT-PFA4, MDT-PFA5, and Ep612 xenograft ependymoma models (Donovan et al., 2020). The trivalent CAR-T therapy also demonstrated significantly increased survival in two medulloblastoma PDX models (MED114FH and Med411FH), but not in an MDT-MMB model. However, EPHA2 monovalent CAR-T therapy also demonstrated increased survival in all three of these medulloblastoma PDX models.
HER2 monovalent and trivalent CAR-T therapies, as well as HER2-specific CAR-macrophages, have shown strong preclinical efficacy in multiple ependymoma and glioma models:

- HER2 CAR-T therapy has shown significant tumor reduction in SU-DIPG36 glioma bearing mice (S. S. Wang et al., 2023)
- Trivalent (HER2, IL13Rα2, and EphA2) T-cell therapy has better efficacy in tumor reduction and prolonged survival compared to single CAR (IL13Rα2) and bi CAR (IL13Rα2 and EphA2) in two orthotopic PDX model (UPN 001 and UPN005) (Bielamowicz et al., 2018)
- DNA nanocarriers can efficiently reprogram tumor-associated macrophages to Her2-specific CAR-macrophages in orthotopic brain-stem glioma PDX model to reduce tumor burden and prolong survival (L. Gao et al., 2023).

Preclinical Safety and Toxicology
HER2 gene disruption and drug targeting is associated with significant risk of cardiotoxicity, which can be mitigated with cardioprotective agents or novel drug modalities. Her2 KO mice die early in embryonic development due to dysfunctions associated with a lack of cardiac trabeculae (Lee et al., 1995). Severe defects in development of sensory and motor neuronal systems were also reported, highlighting the importance of HER2 to early-stage cardiac and neural development. Conditional knockout models have been useful for distinguishing tissue-specific pathologies in Her2 KO mice. Disruption of Her2 expression in ventricular cardiomyocytes led to severe dilated cardiomyopathy, with signs of cardiac dysfunction generally appearing by the second postnatal month (Özcelik et al., 2002). Moreover, rescuing cardiac defects in KO mice revealed sustained sensory and motor deficits and perinatal lethality (Morris et al., 1999).

For Herceptin (Genentech-produced trastuzumab), significant cardiac toxicity has been reported in adult patients, particularly in anthracycline-based trastuzumab regimens. However, a phase II trial of trastuzumab and anthracycline-based chemotherapy in pediatric osteosarcoma patients found no evidence of acute cardiotoxicity when patients received the cardioprotective agent dexrazoxane (Ebb et al., 2012). In contrast to Herceptin, multiple late-stage trials of Enhertu (Daiichi Sankyo-produced T-DXd) in adult cancer patients have shown no significant cardiotoxicity (Goto et al., 2023; Modi et al., 2022); ADC therapies generally have improved clinical safety profiles due to reduced off-target activity.

Clinical Trial Development
Historic trials of the HER2 inhibitor lapatinib in pediatric CNS malignancies were well tolerated but showed little single agent activity (Fouladi et al., 2013). Notable recent clinical trials are summarized below.

Phase II Trials
The following recent phase II trials include pediatric patients:
• **Trastuzumab deruxtecan in HER2-positive osteosarcoma**: This study is evaluating the efficacy and safety of T-DXd in treating patients 12 to 39 years old with HER2-positive osteosarcoma that is newly diagnosed or recurrent. T-DXd did not demonstrate sufficient response to expand enrollment to the planned second stage (Reed et al., 2023). No patients had significant HER2 membranous staining which may account for the limited efficacy. No new toxicities were observed. Further correlative studies (i.e., pharmacokinetics [PK], anti-T-DXd analyses, and ctDNA are ongoing (NCT04616560).

• **Osteosarcoma maintenance therapy with Lovaxin B**: This study is evaluating EFS of Lovaxin B therapy in patients 12 to 39 years old with HER2-positive osteosarcoma that recurred in the lungs and has recently been surgically resected. The study is currently recruiting, but no results are available (NCT04974008).

• **Trastuzumab with chemotherapy in AML or solid tumors**: This study is evaluating the feasibility and EFS of trastuzumab in combination with chemotherapy in patients 0 to 18 years old with AML or solid tumors. The study is currently recruiting, but no results are available (NCT02638428).

**Phase I Trials**
The following recent phase I trials include pediatric patients:

• **Neratinib in solid tumors or hematologic malignancies**: This is a dose-finding study of neratinib in patients 3 to 21 years old with relapsed/refractory (r/r) solid tumors or hematologic malignancies, including leukemias and lymphomas. The study is currently recruiting, but no results are available (NCT02932280).

• **HER2 CAR-T therapy with checkpoint blockade in sarcoma**: This is a dose-finding study of an autologous HER2 CAR-T therapy in combination with nivolumab or pembrolizumab in patients 1 to 25 years old with HER2-positive advanced sarcoma, including osteosarcoma, rhabdomyosarcoma, Ewing sarcoma, and synovial sarcoma. The study is currently recruiting, but no results are available (NCT04995003).

• **HER2 CAR-T therapy in r/r CNS tumors**: This study is evaluating adverse events (AEs) of an autologous HER2 CAR-T therapy in patients 1 to 26 years old with HER2-positive pediatric CNS tumors, including glioma, ependymoma, medulloblastoma, and pineoblastoma. The study is currently recruiting, but no results are available (NCT03500991).

• **HER2 CAR-T therapy in ependymoma**: This is a dose-finding study of an autologous HER2 CAR-T therapy in patients 1 to 22 years old with HER2-positive r/r ependymoma. The study is currently recruiting, but no results are available (NCT04903080).

**Research and Development Landscape**
Focus on HER2 has remained consistently high since peaking in 2013, with strong interest surrounding HER2 as a target in oncology. However, preclinical studies of HER2 as a pediatric oncology target are infrequent. Glioma is the most referenced indication in HER2 literature, with approximately twice as many references as any other indication.
There are 34 approved HER2-targeted products worldwide. Six assets are currently under active investigation in clinical trials for key pediatric cancer indications; Herceptin, Enhertu, and Lovaxin B have advanced to phase II trials, while others are in phase I. The global pipeline of HER2-targeted products is rich, with 99 assets involved in clinical oncology programs that have advanced to phase I pre-registration.

**Additional Considerations for Preclinical and Clinical Development**

*Facilitated by Filemon Dela Cruz, MD, Memorial Sloan Kettering Cancer Center, and Michael Ortiz, MD, Memorial Sloan Kettering Cancer Center*

**MOAs for HER2-Targeting Agents**

Currently, HER2-targeting agents have multiple MOAs: HER2 kinase activity inhibition, HER2 mAbs acting through blocking intracellular signaling and antibody-dependent cell-mediated cytotoxicity (ADCC), HER2-targeting ADCs using various payloads with differing MOAs (e.g., topoisomerase 1 [TOP1] inhibition), and HER2-targeting CAR-T cell therapies. HER2-targeting ADCs and CAR-T cell therapies have shown promise in preclinical studies and adult clinical trials. However, the pediatric oncology community has shown low interest in developing agents to inhibit HER2 kinase activity, in part due to the potential for HER2 kinase inhibitors, when used in combination therapeutic approaches, to potentially alter the activity of HER2-targeting ADCs or CAR-T cell therapies. Thus, future preclinical and clinical studies should focus on understanding the activity of HER2-targeting ADCs and CAR-T cell therapies in HER2-expressing pediatric indications. Specifically, HER2-targeting ADCs have shown anti-tumor activity in preclinical studies of Wilms and rhabdoid tumors, which may warrant clinical testing.

**HER2-targeting ADCs**

T-DXd, a HER2-targeting ADC carrying a TOP1 inhibitor payload, has shown promise in preclinical studies and adult clinical trials. Notably, the phase 2 DAISY and DESTINY trials reported anti-tumor activity of T-DXd in HER2 low- and non-expressing adult metastatic breast cancer. However, it remains unclear whether the effectiveness of T-DXd is due to successful HER2 targeting for payload delivery or a bystander effect (i.e., systemic, non-targeted exposure to the payload). Several ongoing studies evaluating HER2-targeting ADCs in preclinical models are comparing treatment efficacy in HER2-positive and HER2 low- or non-expressing tumors. These ongoing studies, along with studies comparing HER2-targeting ADCs with different payloads, may provide additional insight into the MOA of HER2-targeting ADCs. In addition, a basket clinical trial studying the efficacy of T-DXd in treating tumors with high HER2 expression could provide similar insights; if HER2 high-expressing tumors do not respond to T-DXd, this may indicate that HER2-targeted payload delivery may not contribute significantly to T-DXd efficacy.

**HER2 Detection**

Using IHC, HER2 expression has been observed both on the tumor cell surface and in the cytoplasm, primarily in the epithelial tubular and blastemal components of tumors. However, HER2 IHC findings are difficult to interpret, especially in HER2 low-expressing histologies, due to variability in HER2 detection using different HER2 antibodies. Several studies have used ERBB2
mRNA expression as a surrogate for HER2 protein expression, but it remains unclear whether low HER2 detection using IHC and mRNA assays accurately reflects low HER2 expression or that the assays are not sufficiently sensitive to detect HER2 expression. Neither ERBB2 mRNA levels nor HER2 IHC levels correlated with anti-tumor activity of T-DXd in PDX models.

**Next Steps**

In summary, HER2 is a low-to-moderate priority target—scoring 2.8 out of 5 in the online poll—based on the need for additional clinical trials studying the efficacy of HER2-targeting ADCs and CAR-T cell therapies, rather than additional preclinical studies. A pediatric basket clinical trial of T-DXd in HER-2 high-expressing tumors could help to determine whether targeting HER2 is relevant to the anti-tumor activity of HER2-targeting ADCs, and there is interest in evaluating T-DXd for Wilms tumor and rhabdoid tumors. However, additional preclinical studies can provide insights into MOAs of HER2-targeting agents and methods of enhancing HER2 detection via IHC.
Appendix A: Feedback and Next Steps

Ms. Tetyana Murza and Dr. Stacey Adam facilitated a feedback session with meeting participants to discuss the pre-meeting materials, COACH meeting format, target selection process, and continuation of COACH meetings. Meeting participants appreciated that pre-meeting materials provided thorough summaries of each pediatric cancer target and found the meeting format acceptable. FNIH implemented several suggestions from the COACH email survey to modify the meeting format for the Seventh Quarterly Collaboration Meeting, including shortening the introduction of each target and focusing discussions on preclinical opportunities. Meeting participants found the target selection process acceptable and appreciated the use of live polling during the meeting to vote on future targets.

The stakeholders provided varying reviews on the impact of COACH on their work; however, many felt it would be worth pursuing discussions about an extension to the meetings if there were some format changes incorporated. The stakeholders detailed recommendations on these updates. Most partners did conclude that now would not be the best time to revisit the full larger partnership, ACT4PEDS, that COACH was originally part of, but rather felt further discussions in the future would be more fruitful.

The final COACH Quarterly Collaboration Meeting will occur on March 11, 2024, from 10:00 am to 2:00 pm Eastern Time. Meeting participants voted on which of the remaining targets to discuss during the next meeting. Src homology 2 containing protein tyrosine phosphatase 2 (SHP2) and glypican 3 (GPC3) received the highest number of votes, with several meeting participants also recommending that multiple glypicans be discussed rather than just GPC3. Meeting participants did not favor discussing cyclin-dependent kinase (CDK) targets, which were reviewed during the ACCELERATE Pediatric Strategy Forum in October 2023. FNIH will gather feedback from meeting participants and identify a third target before the March meeting.


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