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ABSTRACTS

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Non-myogenic origin of embryonal rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children. Despite aggressive chemotherapy, radiotherapy and surgery, clinical outcomes for RMS have not improved for three decades, emphasizing the need to uncover the molecular underpinnings of the disease. RMS includes two histolopathologic subtypes: alveolar RMS, driven by the fusion protein PAX3/7-FOXO1, and embryonal RMS (ERMS), which is genetically heterogeneous. RMS has been presumed to originate from derailed muscle progenitors based on the histologic appearance and gene expression pattern of the tumors. However, an origin restricted to skeletal muscle does not explain RMS occurring in tissues devoid of skeletal muscle such as the prostate, bladder, biliary tree and the omentum.
Previously, we showed that activation of Sonic Hedgehog signaling through expression of a conditional, constitutively active Smoothened allele, *SmoM2*, under control of an adipocyte-restricted adipose protein 2 (aP2)-Cre recombinase transgene in mice gives rise to aggressive skeletal muscle tumors that display the histologic and molecular characteristics of human ERMS. In this model, tumorigenesis occurs with high penetrance (~80%), is early onset (by 2 months of age), and is restricted to the head and neck. Also, unlike previous RMS models, this model requires no additional background mutations, such as inactivation of *p53*, and results in only ERMS neoplasia. We illustrated that the gene expression signature of the *aP2-Cre;SmoM2* tumors recapitulates both other mouse ERMS models as well as human ERMS.
With the short latency and anatomic restricted tumor location, we sought to leverage this model to explore the cell of origin. Lineage tracing the *aP2-Cre* in combination with reporter mice illustrated aP2-Cre expression in both brown and white adipose tissue as well as a discrete population of cells lying between skeletal muscle fibers but not beneath the laminin sheath of the muscle fibers. These aP2-lineage cells are distinct from Pax7-positive skeletal muscle stem cells or satellite cells and do not contribute to myofiber formation. When compared to *aP2-Cre;R26-Tom* mice, the addition of oncogenic SmoM2 (*aP2-Cre;R26-Tom;SmoM2*) results in embryonic expansion of the aP2-lineage interstitial muscle cells and formation of ERMS. Our findings suggest that non-skeletal muscle progenitors are a potential cell of origin for Sonic Hedgehog-driven ERMS.

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A transposon screen identifies loss of primary cilia as a mechanism of resistance to Smo inhibitors

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Targeted cancer therapies promise high efficacy with limited toxicity. However, acquired resistance to targeted therapies frequently results in tumor recurrence. Mutations of the therapeutic target itself represent one mechanism for resistance, however the broad scope of changes that confer resistance is not known. Aberrant Hedgehog signaling is implicated with many cancers. It is particularly evident in medulloblastoma, the most common malignant brain tumor in children. Preclinical and clinical studies have demonstrated that medulloblastoma exhibit partial or complete responses to Smo inhibitors. However, clinical benefits are limited by de novo or acquired resistance. Identification of resistance mechanisms is essential to overcome resistance and to achieve long-term benefits. Here we carried out a transposon mutagenesis screen in Hedgehog-pathway dependent medulloblastoma cells, and identified mutations in genes essential for primary cilia formation conferring resistance. Analysis of clinical samples indicates loss of primary cilia representing a new class of resistance mutations. This was extremely surprising, as loss of primary cilia is predicted to inhibit growth of Hedgehog pathway-dependent tumors, rather than to confer resistance. We demonstrate that loss of primary cilia confer therapeutic resistance by initiating a persister state, in which slow growing tumor cells maintain a low level of Gli2-dependent transcriptional output in the presence of targeted therapies. Additionally, synergy between cilia loss and heterozygous mutations in tumor suppressor genes can transform slow growing persister cells into rapidly growing tumors. We examined clinical samples and found that loss of cilia is a common feature of resistant tumors. Together these findings reveal novel mechanisms of resistance and identify consistent strategies for treating diverse resistant tumors.

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Genomic landscape of infant acute lymphoblastic leukemia with *MLL* (*KMT2A*) rearrangement

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Acute lymphoblastic leukemia in infants <12 months of age is an aggressive cancer with high risk of relapse. Infant ALL with *MLL* (*KMT2A*) translocation (MLL-R) has an event-free survival of <50% and the prognosis following relapse is dismal. Other than MLL-R, infant ALL contains remarkably few genomic lesions. We sought to determine if somatic mutations are recurrent among infant ALL cases, if specific mutations may predict resistant disease, and if additional driver mutations emerge at relapse.
Methods: We performed whole genome sequencing (WGS) on blood and bone marrow samples from 25 infants with MLL-R ALL. Cases contained either MLL-AF4 or MLL-ENL. Cohort A included 13 patients with trios of samples from diagnosis, remission, and relapse. Cohort B included 12 non-relapsed patients from diagnosis and remission. WGS was performed using Illumina Hiseq 4000 and 2500, to a minimum of 90Gb. Alignment and variant calling were done using BWA, GATK. Somatic variants were defined as ACMG category 1-3 mutations that were present at diagnosis or relapse, but absent in the remission control, with allelic frequency ≤0.1%, and minimum allelic depth of 7 reads.
Results: In diagnostic samples, we identified 57 non-silent somatic mutations (43 missense, 7 frameshift, 5 nonsense, 1 mitochondrial, and 1 indel) among 48 genes. Recurrent mutations at diagnosis occurred in *NRAS* (N=5), *KRAS* (N=3), *PIK3R1* (N=2), and *PI3KCD* (N=2). Mutations in *NRAS* or *KRAS* were found in MLL-AF4 cases only. In relapse samples, the number of non-silent mutations increased to 132 (97 missense, 13 frameshift, 9 nonsense, 1 mitochondrial, 3 indel, and 9 spice variants). The mean number of mutations per case increased from 2.2 ± 1.3 at diagnosis to 10 ± 12 at relapse. The median number increased from 2 (range 0-4) at diagnosis to 7 (range 0-44) at relapse. On average, 8.8 mutations were gained (range 0-40), 1.3 were retained (range 0-4) and 0.8 were lost (range 0-3) at relapse. Variant allele frequencies were 0.35 ± 0.17 at diagnosis and 0.38 ± 0.14 at relapse for Cohort A and 0.42 ± 0.12 at diagnosis for Cohort B.
Conclusions: In the largest series of infant ALL trio sequencing to date, we found a paucity of pathogenic mutations at diagnosis or relapse. While *NRAS* and *KRAS* mutations were recurrent, they were present in only 21% of cases. Our findings suggest that epigenomic, rather than genomic, factors may drive chemotherapy resistance in MLL-R infant ALL.

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Drug conjugated nanoparticles activated by cancer cell specific mRNA

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We have developed a customizable approach to cancer therapy in which a gold nanoparticle (Au-NP) delivers a drug that is selectively activated within the cancer cell by the presence of an mRNA unique to the cancer cell. Fundamental to this approach is the observation that the amount of drug released from the Au-NP is proportional to both the presence and abundance of the cancer cell specific mRNA in a cell. Concurrent with drug release, the mRNA bound to the Au-NP also undergoes degradation by nucleases targeting DNA/RNA hybrids and, therefore, depletes the cancer cell of a gene required for survival and proliferation. This provides a novel targeting opportunity to dramatically increase the concentration of free drug in cancer cells relative to normal cells and, therefore, maximize efficacy and minimize toxicity while simultaneously depleting the cancer cell of an essential mRNA. This approach is also highly customizable with respect to both the cancer cell specific mRNAs targeted and drugs activated and, thus, has broad applicability across cancer. As proof-of-principle, we have demonstrated both the efficient delivery and selective release of the multi-kinase inhibitor dasatinib from Au-NPs in leukemia cells with resulting efficacy *in vitro* and *in vivo*. Furthermore, these dasatinib-conjugated Au-NPs reduced toxicity against hematopoietic stem cells and T-cells. We are now expanding this technology and its therapeutic potential by utilizing SN38, the highly potent metabolite of the topoisomerase I inhibitor irinotecan that is used in the therapy of multiple cancers. SN38 is an ideal drug for this approach as although it is 100-1000 fold more potent than its pro-drug irinotecan, only a small fraction of irinotecan is metabolized to SN38 in patients and it cannot be given clinically due to insolubility. We are currently testing SN38-conjugated Au-NPs with Ewing sarcoma and neuroblastoma cells. This work will provide a strong foundation for both moving this novel cancer therapy approach closer to the clinic and developing more complex Au-NPs that selectively activate two or more synergistic drugs or are functionalized with additional biologic agents to enhance delivery or efficacy.

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5p-derived microRNAs inhibit Wilms tumor growth

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Wilms tumor is the most common kidney tumor of childhood and the third most common pediatric solid tumor overall. One-fifth of Wilms tumors are driven by mutations in the enzymes responsible for microRNA processing, notably *DROSHA*, *DICER1*, and *DGCR8*. microRNAs regulate gene expression post-transcriptionally in a sequence-specific manner, and dysregulation of microRNAs is seen in many cancers. Specifically, mutations in microRNA processing genes are common in a variety of cancers, including pleuropulmonary blastoma, pineoblastoma, and Sertoli-Leydig cell tumor. These mutations act in different ways to block the biogenesis of tumor-suppressing microRNAs, especially those derived from the 5p arms of pre-microRNA hairpins. This implies that 5p-derived microRNAs play an important tumor-suppressive role – a role which may lead to a new therapeutic opportunity, since microRNAs can be pharmacologically re-introduced. For this reason, we sought to test whether re-introduction of specific 5p-derived microRNAs could be a new treatment strategy for Wilms tumors.
However, the most important 5p-derived microRNAs to therapeutically replace in this context are unknown. To identify such microRNAs, we analyzed microRNA expression in Wilms tumors bearing mutations in microRNA processing genes. We found that the let-7, miR-16, and miR-34 families are particularly deficient in Wilms tumors with microRNA-impairing mutations. These 5p-derived microRNAs are also key tumor suppressors in other cancers, and liposomes designed to deliver these microRNAs are in development for cancer therapy. To study their impact on cell proliferation in the context of Wilms tumor, we used a lentiviral system for inducible expression of each microRNA at physiologically relevant levels. Specifically, we expressed let-7a, miR-16, and miR-34a in two Wilms tumor cell lines, WiT-49 and WT-CLS1. Expression of each of these microRNAs resulted in repression of target genes and resulting growth suppression. This suppression occurred through both decreased proliferation and increased apoptosis. In both cell lines, miR-16 and miR-34a induction had a larger impact on growth than let-7a. Thus, miR-16 and miR-34a are promising microRNAs for pharmacological re-introduction as a novel therapy for Wilms tumors and other cancers defective for these microRNAs.

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Molecular profiling using targeted next-generation sequencing in pediatric neuro-oncology patients: the UCSF experience

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Background: Molecular profiling is revolutionizing cancer diagnostics beyond morphology and immunohistochemistry and leading to personalized therapeutic approaches. Herein, we describe our institutional experience performing targeted sequencing for 40 pediatric neuro-oncology patients.
Methods: We sequenced approximately 500 cancer-associated genes in DNA extracted from micro-dissected tumor tissue and peripheral blood using a standardized bioinformatics pipeline to identify germline and somatic mutations and copy number changes. Patients were selected from the primary UCSF population or referrals from outside institutions. Cases selected for sequencing were chosen according to diagnostic uncertainty, diagnoses without successful standard of care therapy, or recurrent or treatment-refractory tumors. Results were discussed at a standing multi-disciplinary molecular tumor board to identify clinically relevant alterations and potential therapy options.
Results: Between June 2015 and August 2016, genomic profiling was performed on 41 brain tumors from 40 pediatric patients, including 7 low-grade gliomas, 15 high-grade gliomas, 10 medulloblastomas, 2 high-grade neuroepithelial tumors, 1 CNS neuroblastoma, 1 embryonal tumor with multilayered rosettes, 1 pineoblastoma, 1 uveal ganglioneuroma, 1 chordoma, 1 meningioma, and 1 choroid plexus carcinoma. One patient had sequencing completed on 2 apparently de novo glioblastomas as based on molecular profiling. In 32 cases (80%), results impacted patient management by 1) clarifying diagnosis, 2) identifying previously unsuspected pathogenic germline mutations, or 3) detecting potentially targetable somatic alterations. Nine pathologic diagnoses were amended based on genomic profiling results, including a high- to low-grade glioma, medulloblastoma to pineoblastoma, and ependymoma to CNS high-grade neuroepithelial tumor with BCOR alteration. Ten patients were identified to have previously

unsuspected pathogenic germline mutations including 2 patients with high-grade gliomas harboring novel *MUTYH* germline mutations and one patient found to have both *TP53* and *MSH6* germline mutations consistent with concurrent Li-Fraumeni and Lynch syndromes. Potentially targetable mutations were identified in 24 patients (63%) including three patients with hypermutated glioblastomas, recently identified to therapeutically benefit from PD-1 inhibition. Additionally, novel, likely pathogenic alterations were identified in three cases: an in-frame *RAF1* fusion in a *BRAF* wild-type pleomorphic xanthoastrocytoma, an inactivating *ASXL1* mutation in a histone H3 wild-type diffuse pontine glioma, and an in-frame deletion within exon 2 of *MAP2K1* in a low-grade astrocytic neoplasm.
Conclusions: Our experience demonstrates the significant impact of molecular profiling on diagnosis and treatment of pediatric brain tumors and confirms its feasibility for use at time of initial diagnosis or recurrence.

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Comparative cancer genomic analysis nominates novel therapeutic options for individual pediatric cancer patients

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The Cancer Genome Atlas (TCGA) and Therapeutically Applicable Research to Generate Effective Treatments (TARGET) projects have produced genomic data from thousands of adult and pediatric tumors. However, it is unclear whether these datasets could directly benefit children with cancer today. UC Santa Cruz Treehouse Childhood Cancer Initiative (treehouse.soe.ucsc.edu) enables comparisons of genomic information from children with cancer treated on clinical genomic trials to previously collected cancer genomic datasets. Through this approach, termed “pan-cancer analysis”, we aim to identify molecular features that may suggest new therapies for children with cancer.
In our pan-cancer analysis, each tumor’s RNA-sequencing profile and/or mutational profile is compared to 10,368 uniformly analyzed tumor profiles from TCGA and TARGET projects (Vivian et al., BiorXiv, 2016). We use Tumor Map, an unsupervised clustering and visualization tool, to identify tumors in the reference cohorts that are most similar to the given tumor. We then perform a gene expression outlier analysis that reveals transcripts, whose expression is significantly enriched or depleted in the given tumor. These data are used to generate hypotheses regarding the molecular pathways that may be driving the disease in each child as well as relevant targeted therapies, providing clinical directions that could be further investigated by the medical teams.
We have been evaluating the Treehouse analysis approach as part of California Kids Cancer Comparison, a demonstration project for the California Initiative to Advance Precision Medicine. With the generous funding from St. Baldrick’s Foundation, we are expanding our study to include children treated on clinical genomic trials at Stanford University, Pacific Pediatric Neuro-Oncology Consortium, Children’s Hospital of Orange County, University of Michigan, Children’s Hospital of Philadelphia, and British Columbia Children’s Hospital. The analysis of the first 19 patients at Stanford provides evidence of the potential clinical utility of our approach. In 17 of the 19 cases, we found rationale for new therapeutic options, including existing clinical trials, and/or FDA-approved drugs that could be administered off-label at the discretion of the physician. In 2 patients, both of whom had no available treatment options prior to our work, the comparative analysis has contributed to treatment decisions. This study provides a framework for using large, public genomic datasets to help interpret genomic information from prospective pediatric cancer patients. Our study also underscores the importance of releasing cancer genomic datasets to the community immediately following data generation, so that they may be used to benefit new patients.

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OTX2 activity at distal regulatory elements shapes the chromatin landscape of Group 3 medulloblastoma

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Medulloblastoma is the most frequent malignant pediatric brain tumor and is divided into at least four subgroups. Group 3 patients have the lowest 5-year survival and there is a pressing need to identify oncogenic drivers in these tumors in order to develop novel therapies. Here, we characterized gene regulatory mechanisms in Group 3 tumors through the combined analysis of genome-wide chromatin and gene expression profiling in primary tumor samples and established cell lines. Our results show that the majority of active enhancers in this subtype are occupied by the oncogenic transcription factor OTX2, which is often arranged as clusters of adjacent peaks. Active OTX2 bound enhancers, as opposed to many inactive OTX2 binding sites across the genome, are distinguished by the presence of the transcription factor NEUROD1. These active sites are responsive to OTX2 and NEUROD1 knockdown and could also be generated de novo upon ectopic OTX2 expression in primary cells, showing that OTX2 plays a major role in maintaining and even establishing regulatory elements as a pioneer factor, and that it cooperates with additional factors to shape the regulatory landscape of tumor cells. Among OTX2-regulated target genes we identified the kinase NEK2, whose knockdown and pharmacological inhibition resulted in decreased cell viability. Our studies thus show that OTX2 controls the regulatory landscape of Group 3 medulloblastoma through cooperative activity at enhancer elements and is a major contributor to the expression of critical target genes in this tumor type.

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Functional Multi-omics Identifies Druggable Targets in Renal Medullary Carcinoma

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Renal medullary carcinoma (RMC) is a rare kidney cancer that is primarily seen in adolescent and young adult African American patients with sickle cell trait. Prognosis is poor and treatment options are limited. We have developed one of the first models, CLF-PED-005-M Adherent and Suspension, from a patient who succumbed to this disease in one year's time. We have confirmed by whole exome sequencing that our models have sickle cell trait and loss of heterozygosity of the SMARCB1 loci, both hallmarks of this disease. By RNA-sequencing, we see a lack of SMARCB1 transcription. We have further shown dependency of our models to SMARCB1 re-expression thus suggesting that this cancer is indeed driven by loss of SMARCB1 at a functional level. We performed pooled CRISPR-Cas9 and RNAi loss of function screens and a small molecule screen focused on druggable cancer targets based on our previous work. Integrating these three complementary and orthogonal methods, we identified a number of targets for further validation. These targets, when combined may provide a rational approach to therapeutic targeting for this rare kidney cancer.

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Cavatica: empowering research with a pediatric genomic cloud

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The pediatric cancer genome is severely under-represented in genomic databases as existing data portals have primarily focused on adult cancers. Furthermore, large-scale pediatric datasets like TARGET lack pediatric central nervous system (CNS) data. To address this unmet need, we have developed a new cancer genomic platform named Cavatica. The rationale behind Cavatica is to provide a sustainable application cloud based eco-system that supports many of the aspects associated with basic & translational research. Cavatica is the first of its kind pediatric genomic portal for disease research, and its goal is to serve as a central hub to promote collaborative research between investigators. Cavatica supports the sharing and creation of pipelines, data, algorithms, visualizations, and hypotheses. Currently, one of the biggest barriers and challenges to collaborative research is the transfer and processing of 'big data' such as cancer genomes. By placing data, pipelines, computation, and visualizations on the Cavatica cloud we provide a centralized area for researchers to collaborate on projects and bring their data, algorithms, and expertise. Currently, Cavatica features the following applications designed to work together: a biorepository and specimen query tool (Harvest, harvest.research.chop.edu), a data visualization application (PedcBioPortal, pedcbioportal.org), data storage in S3 buckets, and data processing via Seven Bridges Genomics. Users can move seamlessly between these applications, and thus can go from points on a graph to physical samples. Cavatica also protects data or pipelines on an individual and group basis so various team members can share a common working space with controlled or a single individual can store experiments in a private space. All current solutions will be constantly evaluated and replaced as technology evolves. Cavatica is set to house data from a number of sources including the Childhood Brain Tumor Tissue Consortium (CBTTC), Pacific Neurooncology Consortium (PNOC), Stand Up to Cancer (SU2C), NCI Therapeutically Applicable Research To Generate Effective Treatments (TARGET), and The Cancer Genome Atlas (TCGA). Cavatica's framework will also allow unique opportunities for data scientists, statisticians, data engineers, programmers, application developers, bioinformaticians, and pre-clinical and clinical researchers to contribute and expand the reach and impact of this application.

200: Clinical Pipeline

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PHASE I STUDY OF LOXO-101, A SELECTIVE TRK INHIBITOR, IN PEDIATRIC PATIENTS WITH CANCER

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Background/Objectives: Neurotrophin ligands and their receptors TRKA, TRKB, and TRKC (encoded by *NTRK1, NTRK2, and NTRK3)* are important for growth regulation, differentiation and survival of neurons. Translocations involving the *NTRK1/2/3* kinase domain, mutations involving the TRK ligand-binding site, amplifications of *NTRK*, TRK splice variants, and autocrine/paracrine signaling have been described in diverse tumor types and may contribute to tumorigenesis. A broad range of pediatric malignancies have been found to harbor *NTRK* fusions, including infantile fibrosarcoma (IFS), congenital mesoblastic nephroma (CMN), secretory breast cancer, pediatric papillary thyroid cancer, gliomas and Ph-like acute lymphoblastic leukemia. Additionally, TRK protein over-expression is common in neuroblastoma. LOXO-101 is the first small-molecule selective inhibitor of TRKA, -B, and -C in clinical development and has demonstrated clinically meaningful responses in patients with TRK fusion cancers in an adult phase 1 trial (5/6 patients with RECIST PRs). Design/Methods: We have initiated an open-label, multi-center Phase I dose escalation/dose expansion study with LOXO-101 in pediatric patients with solid tumors and primary CNS tumors (NCT02637687). Patients with advanced cancer between the ages of 1 and 21 years are

eligible, as well as patients as young as 1-month of age with a primary diagnosis of IFS or CMN and a documented NTRK fusion. Twice-daily oral dosing of LOXO-101 capsules is administered on a continuous 28-day schedule. LOXO-101 is available in an oral liquid formulation for patients unable to swallow capsules. Dose escalation utilizes a Rolling 6 design. The objective of the study is to determine the recommended phase 2 dose (RP2D) and initial evidence of the efficacy of LOXO-101 in different tumor types. Eligibility for the dose expansion cohorts will require patient tumor samples to have documented alterations of an *NTRK* gene or TRK protein. Molecular abnormalities will be characterized through the analysis of archival tissue.
Results: This study is ongoing and open for enrollment. The RP2D has not yet been defined. The initial 16-month old patient with an ETV6-NTRK3 fusion positive infantile fibrosarcoma had a rapid radiographic response (PR by RECIST) which has been maintained through 5 cycles of treatment.
Discussion: Abnormalities of the NTRK gene, including translocations, have been identified as possible oncogenic drivers in a number of pediatric cancers. LOXO-101 has demonstrated responses adults and children with NTRK fusion positive cancers. Ongoing research will define the dose of LOXO-101 in children and further evaluate NTRK fusions as a therapeutic target in this diverse population.

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Targeting the chromatin regulatory machinery hijacked by EWS/FLI in Ewing Sarcoma

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Ewing sarcoma is an aggressive pediatric tumor characterized by the expression of the oncogenic transcription factor EWS/FLI. EWS/FLI is a fusion protein resulting from a reciprocal chromosomal translocation, involving chromosomes 11 and 22, t(11;22). Apart from this pathognomonic lesion, Ewing sarcoma has one of the lowest mutational rates among all cancers (0.15 mutations/Mb). EWS/FLI is a poor candidate for pharmacological blockade due to its intrinsically disordered nature and convex DNA-binding surface. However, EWS/FLI recruits other chromatin regulatory proteins to alter the epigenetic landscape of Ewing sarcoma cells and maintain malignancy, and these factors represent potentially actionable targets. We have identified one such protein, lysine specific demethylase 1 (LSD1), whose depletion and pharmacological inhibition dramatically impair Ewing sarcoma cell viability. Every Ewing sarcoma cell line in our comprehensive panel (n= 17) displayed sensitivity to benzohydrazide-mediated LSD1 inhibition. LSD1 inhibition with HCI2509 compared to tranylcypromine (TCP) and other TCP derivatives currently in clinical trials demonstrated class-dependent derepression of EWS/FLI-repressed genes. Moreover, mutation of LSD1 in Ewing sarcoma patient tumors was not observed in a meta-analysis of five whole-genome and whole-exome sequencing studies.
The mechanistic details by which LSD1 and EWS/FLI interact remain undefined. In order to test how LSD1-containing complex composition and function are affected by EWS/FLI, we performed co-immunoprecipitation (co-IP) experiments and mass spectrometry to identify how LSD1 protein-protein interactions change in the context of EWS/FLI depletion. Co-IP to mass spec experiments showed several known interactors of LSD1, including CoREST1 and CoREST3, members of the nucleosome remodeling and deacetylase (NuRD) complex, members of the BRAF35 complex, and ZMYM2. Further validation experiments show that EWS/FLI alters the populations of LSD1 protein in the nucleus in "knockdown-rescue" experiments, suggesting an upstream role for EWS/FLI in the regulation of LSD1 in Ewing sarcoma cells. We conclude the LSD1 complexes identified remain relatively stable in Ewing sarcoma with EWS/FLI depletion. However, other facets of LSD1-containing complex biology are regulated by EWS/FLI and the mechanisms by which this occurs remain an area of active study. Understanding these mechanisms and how they relate to the susceptibility of Ewing sarcoma cells to LSD1 inhibition will facilitate rational clinical use of LSD1 inhibitors, not only in Ewing sarcoma, but in other pediatric solid tumors.

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Targeting of WEE1 in Myc-driven medulloblastoma

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Background: Medulloblastoma (MB) is the most common malignant pediatric brain tumor and high-risk patients continue to have poor outcome, with only a 30% survival at 5 years. The Myc oncogene is amplified or overexpressed in about half of this patient population. One potential therapeutic strategy is to exploit Myc oncogene driven replicative stress by inhibiting WEE1, a kinase known to participate in the G2-M cell cycle checkpoint and DNA replication during the S-phase. Recent data from our lab have identified WEE1 as a critical mediator for medulloblastoma cell viability.
Hypothesis: We hypothesize that WEE1 plays a critical role in Myc-driven MB by protecting cells from oncogene-induced replicative stress and promoting DNA damage repair. With this logic, high Myc expressing tumors will be exquisitely sensitive to AZD-1775, a WEE1 small molecule inhibitor. We further hypothesize that combination of AZD-1775 with DNA damaging agents would be a very promising therapeutic strategy.
Methods/Results: Using commonly used MB cell lines, including primary patient derived cell lines, we showed that sensitivity to AZD-1775 correlated with Myc expression levels. Enhanced expression of Myc in retinal pigment epithelial (RPE) cells, made cells exquisitely sensitive to AZD-1775. A high throughput screen of 91 FDA-approved cytotoxic chemotherapy agents identified inhibitors of the replication machinery and nucleotide synthesis to be strongly synergistic with AZD-1775, with gemcitabine having the highest index. Treatment of cells with AZD-1775 and gemcitabine confirmed synergy in killing cells using the Chou-Talalay method. Combination of these two drugs was also synergistic in causing apoptosis, senescence, and an increase in markers of DNA damage. Treatment with these two drugs also caused a markedly abnormal cell cycle distribution and a decrease in DNA replication. In addition, immunohistochemical analysis of patient samples established that elevated WEE1 expression in group 3 high cMYC expressing tumors conveys a worse clinical outcome. A murine orthotopic xenograft model is currently underway to prove the combination of AZD-1775 and gemcitabine *in vivo*. This project proposes changing the paradigm of MB therapy as it identifies a subgroup of patients that will most likely benefit from new targeted therapy.

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Anti-tumor effect of trastuzumab, GM-CSF and IL-2 combinatorial immunotherapy in preclinical model of high-risk pediatric ependymoma

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A number of studies have demonstrated correlation of host immune factors with outcome in ependymoma (EPN). This supports the development of immunotherapy for EPN, a tumor in which approximately 50% of patients suffer recurrence and for which chemotherapy has not yet shown any benefit. Mechanisms of therapeutic antibody effect in cancer treatment have been shown to include recruitment of host anti-tumor immunity, and may therefore provide an expedient immunotherapeutic approach for EPN. Using a transcriptomic screen of FDA-approved therapeutic antibody targets we identified ERBB2, targetable by therapeutic antibody trastuzumab, as overexpressed in all subgroups of EPN, which was subsequently confirmed by protein analysis. In a series of preclinical studies, we evaluated the combination of therapeutic antibody trastuzumab with immunostimulatory factors GM-CSF and IL2 for the treatment of EPN. Novel EPN 1q+ cell lines 811 and 928 were co-cultured with autologous peripheral blood immune cells to measure immune-cell mediated cytotoxicity using a live cell imaging system (Incucyte). This demonstrated that trastuzumab could effectively target EPN through antibody-dependent cell-mediated cytotoxity (ADCC). Further, this mechanism could be enhanced by combination of GM-CSF with trastuzumab treatment, resulting in increased cytotoxicity. This implicates monocyte/macrophages as effectors of trastuzumab-dependent ADCC in EPN. Addition of IL-2 to trastuzumab and GM-CSF also resulted in further increases in ADCC, suggesting involvement of T-cells. Primary human EPN organotype culture studies demonstrated decreased tumor proliferation and increased immune cell proliferation in response to combined trastuzumab, GM-CSF and IL2. As the first stage in a clinical trial of trastuzumab in recurrent EPN we have recruited patients to a pilot study of GM-CSF delivered prior to surgery. Post-treatment tumor samples demonstrated upregulated antigen processing and presentation genes, a hallmark of GM-CSF activated macrophage/monocyte immunophenotype. These results support the continued testing of GM-CSF in combination with trastuzumab in EPN. Addressing, in part, blood brain barrier penetrance issues, we identified measurable levels of trastuzumab in EPN tumor post-treatment in two clinical cases. Collectively these preclinical and clinical data support continued testing of trastuzumab-based immunotherapy for recurrent EPN.

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Biological response modifiers with metronomic chemotherapy backbones as biomarker-driven add-on treatment strategy in very high risk pediatric solid tumors and NHLs – a single institution pilot study

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Most current anti-cancer therapies are designed with the goal of disease eradication. Although significant progress has been made using this strategy in pediatric ALL, the same cannot be said for histologically and genetically more complex solid tumors. Yet, in cases where chemotherapy with or without radiation does not achieve elimination of all tumor cells, the use of high dose chemotherapy and radiation selectively kills those cells that are chemo-sensitive and leaves highly resistant cancer cell populations free to relapse. Despite the initial reductions in tumor bulk produced by chemotherapy/radiation, the tumors are often quickly re-populated by the resistant cancer population. The purpose of this pilot study was to evaluate toxicity, safety and efficacy of Biomarker-Driven Molecularly-Targeted Metronomic Chemotherapy in n=1 trial setting.
Theranostic approach was tested in real life clinical situation. During 9/2014 – 6/2016 86 children with relapsed, refractory or very high risk solid tumors and NHLs were enrolled. Tumors were assessed using NGS TruSight Tumor panel with 26 most common genes, and where the frozen/fresh tissue was available, whole transcriptome analysis and analysis of RTK and downstream signaling pathways using phosphoprotein arrays were performed. In high-risk neuroblastoma patients, we also analyze expression of selected candidate marker proteins (PBX1, HOXC9, HMGA1, HMGA2 and DDX39A) that may predict resistance or sensitivity to the treatment with retinoids.
While using TruSight Tumor, 31 mutations was found in 25 children, with 3 of them as germline. Other germline mutations were proven in 4 other patients in this cohort using candidate gene approach based on mostly phosphoprotein array data. Theranostic approach combining DNA NGS panel, whole transcriptome and phosphoproteomic data are combined with conventional histopathology data and discussed in multidisciplinary molecular oncology tumor board. This data set led to proposal for personalized treatment for 18 children. The treatment consisted from best matched targeted/ biological treatments /e.g. sunitinib, axitinib, nivolumab, or repositioned drugs combined up to 4 such agents with low dose metronomic chemotherapy backbone. The treatment was very well tolerated, however with some severe, despite transitory toxicity. Best therapeutic results were observed in cases, where phosphoproteomic, transcriptomic and genomic data were matching. Surprising and durable responses were observed e.g. in cases of chemorefractory relapsed Burkitt lymphoma, DIPG, GBM, relapsed anaplastic

ependymomas and low grade sarcomas with underlying germline abnormities.
Our data are showing feasibility of such approach with promising preliminary data however meticulous follow up of children on new targeted therapies remains necessary.
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Rare but recurrent intrachromosomal 6q22 microdeletions generate targetable ROS1 oncoproteins in glioblastoma and ependymoma

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Brain tumors are the leading cause of cancer-related deaths in children. Among these, glioblastoma multiforme (GBM) poses substantial clinical challenge, contributing to a significant percentage of brain cancer related mortality in children and adults. Perusal of clinically relevant cancer drivers informs novel molecularly-targeted treatment strategies. Chromosomal rearrangements that generate oncogenic kinase-fusion(s) are promising drug targets and selectively inhibiting them has led to unprecedented tumor responses in several malignancies. Rearrangement involving ROS1, an orphan receptor tyrosine kinase gene, was first described in a GBM cell line in the 1990s. More recently, ROS1-fusions were identified in subsets of diverse pediatric and adult malignancies, including infantile fibrosarcoma, spitzoid melanoma, non-small cell lung cancer (NSCLC) and cholangiocarcinoma. We and others have shown dramatic clinical efficacy of ROS1 kinase inhibitors (ROS1i) in ROS1-fusion expressing patients. Positing that similar benefit could be accomplished for primary brain cancer we re-examined the TCGA glioblastoma dataset using imbalanced 5'/3' exon expression as metric for further wet-lab interrogation of these tumors. Multiple bioinformatics algorithms previously used to interrogate the TCGA datasets had failed to detect ROS1-fusions in GBM. Here we report the discovery of rare (frequency = 0.5-1%) but recurrent intra-chromosomal 6q22 microdeletions that generate ROS1-fusion proteins in GBM patient samples. Examination of the MSK-IMPACT (MSKCC) and Foundation Medicine genomic sequencing datasets validated the finding that GOPC-ROS1 is a recurrent ROS1-fusion in glioblastoma. We provide the first evidence of GOPC-ROS1 expression in pediatric glioblastoma and ependymoma. Our findings underscore the need for specific clinical tests designed to identify these rare, but important somatic aberrations. To gauge the importance of ROS1-fusions for glioma growth, we performed functional studies assessing their transforming potential using Ba/F3 cells and human astrocytes. Our results demonstrate that ROS1-fusions are dominant oncogenes driving cytokine-independent Ba/F3 cell growth and anchorage-independent colony formation in astrocytes. We then examined the clinical translation potential of targeting ROS1-fusions in GBM using U118MG, a human GBM cell line harboring native GOPC-ROS1 and show that multiple ROS1i effectively inhibit U118MG 3D-spheroid growth by downregulating critical cell growth and survival pathways mediated by the SHP2/RAS/MAPK and PI3K/AKT signaling axes. Additionally, we demonstrate that oral monotherapy with the brain-permeable ROS1i, lorlatinib, effectively reduces U118MG tumor volume and prolongs survival in an intra-cranially xenografted model of the disease. Taken together, these data strongly suggest that the identification and targeting of ROS1-fusions will permit precision oncology and improve outcome in a subset of adult and pediatric glioblastoma patients.

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Radiosensitization of medulloblastoma by a novel DNA damage checkpoint inhibitor

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Medulloblastoma is a cerebellar tumor and the most common pediatric brain malignancy. Radiation therapy is part of the standard care for this tumor, but its effectiveness is accompanied by significant neurocognitive sequelae due to the deleterious effects of radiation on the developing brain. We have previously shown that the protein kinase MRK/ZAK protects osteosarcoma cells from radiation-induced cell death by regulating cell cycle arrest after ionizing radiation. We now show that siRNA-mediated MRK depletion sensitizes primary medulloblastoma cells to radiation. To translate these findings to the clinic, we set out to develop a small molecule inhibitor of MRK, using as starting point the drug nilotinib, a second generation c-Abl inhibitor that has been shown to be equally effective against MRK. Based on a molecular model of nilotinib bound to the MRK active site, we have derivatized nilotinib to covalently bind to a cysteine in the ATP binding pocket of MRK and showed that this drug, M443, inhibits MRK in an irreversible fashion. Furthermore, M443 is highly selective, in that it no longer inhibits c-Abl. We found that M443 strongly radio-sensitizes UW228 medulloblastoma cells as well as IMB226 patient-derived primary cells, but does not radio-sensitize primary human astrocytes or neuronal stem cells. M443 also inhibits radiation-induced activation of both p38 and Chk2, two proteins that act downstream of MRK and are involved in DNA damage-induced cell cycle arrest. Finally, we tested the effect of M443 in an animal model of medulloblastoma that employs orthotopic implantation of IMB226 medulloblastoma cells in nude mice. Intra-tumoral delivery of M443 alone significantly extended animal survival by 5 days compared to vehicle treatment. Combination treatment of M443 with radiation at 2 x 3 Gy, that is not effective on its own (1 day of additional survival over control), achieved a synergistic increase in survival (15 days over the control survival time). Western blotting of tumor lysates with an activation state-specific antibody demonstrated strong inhibition of MRK activity

after M443 administration, thereby validating this antibody as a useful biomarker. A murine model utilizing a paramagnetic gadolinium-based nanoparticle delivery system to bypass the BBB is currently being developed to effectively deliver M443 *in vivo.*
In conclusion, we have developed a new small molecule inhibitor of MRK/ZAK that selectively radio-sensitizes medulloblastoma cells versus normal cells. We hypothesize that combining radio-therapy with M443 will allow us to lower the radiation dose while maintaining therapeutic efficacy, thereby minimizing radiation-induced side effects.

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Evaluation of the dual mTORC1/2 inhibitor TAK228 and MEK inhibitor Trametinib as possible combined treatment for pediatric low grade glioma

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Introduction: Pediatric low grade glioma (PLGG) is one of the most common childhood tumors. If the tumor is located in a region of the brain that is not accessible for surgical resection or if the tumor recurs after surgery, additional therapies are needed. Recent studies highlighted the important role of mTORC and MEK-activation in LGG. The dual mTORC1/2-inhibitor, TAK-228, and the FDA approved MEK-inhibitor, trametinib, have good brain penetration and promising candidates for targeted therapy for LGG.
We hypothesized thatTAK-228 and Trametinib would show synergistic effects both *in vitro* and *in vivo* in PLGG models.
Methods: We treated the PLGG derived cell lines Res186and Res259with TAK-228 (dual mTORC1/2 inhibitor), Trametinib (Mek-inhibitor), DMSO, or TAK-228 combined with Trametinib. Cell growth was investigated using MTT-assay over different days and compared to the treatment with the vehicle. DNA replication was measured through bromodeoxyuridine incorporation assay. Cells were analyzed and counted with ImageJ.
Results: TAK-228 and Trametinib inhibited growth of Res186 and Res259 in a dose dependent manner (50% vs control; $p = 0.01$ as measured by MTT). BrdU incorporation assay revealed a reduction in proliferating cells in 10nM and 100nM Trametinib for Res186 and Res259. Morphological investigation showed an increase in cytoplasm volume with 100nM TAK-228 and Trametinib for both cell lines compared to control groups.
Conclusions and future directions: Our preliminary results show that the PLGG-derived cell lines are sensitive to TAK-228 and Trametinib treatment. All cell lines showed decreased proliferation at various doses of either inhibitor. We will now investigate both drugs *in vivo*as a next step. Evidence of activity in murine models will be necessary to provide a pre-clinical rationale for combination therapy of these agents in aggressive PLGG.

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Pediatric phase 1/1b dose-finding trial of entrectinib with expansion into patients with primary brain tumors, neuroblastoma, and *NTRK*, *ROS1*, or *ALK* fusions

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The STARTRK-NG ("Studies of Tumor Alterations Responsive to Targeting Receptor Kinases – Next Generation") trial is a Phase 1/1b study of oral entrectinib in pediatric patients with relapsed or refractory solid tumors or primary CNS tumors, with two expansion cohorts for subjects with neuroblastoma and other non-neuroblastoma, extracranial solid tumors that harbor a gene fusion in *NTRK1/2/3, ROS1, or ALK.* Entrectinib is a potent inhibitor of solid tumors expressing NTRK1, NTRK2, NTRK3, ROS1 or ALK rearrangements. Gene rearrangements in these genes have been observed in a variety of adult solid tumors, including non-small cell lung and colorectal cancers, and in cancers that affect the pediatric population, including salivary gland cancer, papillary thyroid cancer, melanoma, and sarcomas. In addition, overexpression of TrkB (the protein product of *NTRK2*) and activating *ALK* point mutations have been observed in neuroblastoma. Thus, a pan-Trk, ROS1, and ALK inhibitor like entrectinib may potentially have broad therapeutic utility in pediatric patients.
Phase 1 studies of entrectinib reported a 79% Objective Response Rate in patients with gene fusions of these targets who had not received a previous kinase inhibitor for these genes, received effective therapeutic dose level, and had extracranial primary disease. In addition, a response was seen in a 22-year-old patient with neuroblastoma with an activating point mutation of *ALK*, as well as significant tumor regression of CNS metastases in an 18-month-old boy with infantile fibrosarcoma, harboring an *ETV6-NTRK3* fusion. As of 7 March 2016, a total of 119 patients had been treated with entrectinib in the phase 1 studies. The most common (>10% incidence) treatment-related adverse events were fatigue/asthenia, dysgeusia, paresthesia, nausea, myalgia, diarrhea, dizziness, arthralgia, vomiting, and constipation; importantly, there was no evidence of cumulative toxicity.
Overall, these gene fusions are rare in the cancer population (< 3%); however, they have been seen in over 40 solid tumor histologies. A survey of two pediatric cancer databases, St Jude pediatric cancer database (PeCan; total n=1,604) and the University of Michigan database (Peds-MiOncoSeq; total n=91) resulted in the identification of gene rearrangements in all three *NTRK* genes. In addition, according to a literature survey, the following tumor types, largely confined to the pediatric patient population, are also known to harbor gene rearrangements of the *NTRK* family of genes: congenital or infantile fibrosarcoma, secretory breast cancer, mesoblastic nephroma, and intrinsic pontine gliomas. Thus, targeting Trk receptors with a pan-Trk inhibitor may be of benefit for many cancers in children.

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Germinal mutation of PDGFRalpha in patient with tuberous sclerosis complex

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We are presenting the case report of 9 year old boy with tuberous sclerosis complex. Within that he has angiomyolipomas of kidneys, bilateral hamartomas of retina, pharmacoresistant epilepsy, hypothyreosis. 11/2014 he was diagnosed with malignant PEComa in the abdominal cavity with the residual disease after the surgery.
The mutational analysis from the tumor tissue and then also from the peripheral blood cells proved germinal mutation in PDGFRa – substitution in exon 10. Looking into the literature it remains unclear if this mutation leads to an activation of the protein, but the relationship to the PEComa in this case is suspicious. The analysis of the profile of phosphorylated proteins in the tumor cells revealed highly activated EGFR, InsR, IGF-IR and PDGFRβ.
Due to these results we started with personalized treatment encompassing everolimus/sunitinib/metformin orally: The patient is now without measurable disease according to the ultrasound with EFS/OS 16 months.
The coincidence of tuberous sclerosis complex with another germinal mutation is rare. This case report shows the possibility to use combination of different targeted therapies, which can help to stabilized/cure malignant tumors in such patients.

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Theranostic approach for relapsed APDS like Burkitt lymphoma

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A 7-y old previously healthy boy with no family history of cancer was diagnosed with stage III abdominal Burkitt lymphoma in December 2014. He was initially treated standard BFM B-NHL 04 therapy. After 2 cycles, he had a very good partial response reaching < 5% of the initial tumor volume. An episode of the intestinal obstruction in February 2015 led to excision, and the histology confirmed sclerosing mesenteritis, without histological or rtPCR evidence of lymphoma (the original tissue was positive for cMYC translocation).
Unfortunately the child was found to have an isolated radiological progression in the same region in which the intestinal obstruction had occurred two months after completing chemotherapy. The biopsy in June 2015 confirmed relapsed Burkitt lymphoma, this time with marked areas of sclerosing mesenteritis and mesenteric panniculitis. Mutational analysis of PI3K delta subunit proved germinal mutation/variant C830G at cDNA level and serine 312 cysteine on protein level, which is outside the classical activated PI3K-delta syndrome.
While undergoing the genomic testing, the boy was started on retrieval individualized therapy. It was only when the combination of CyVe cycle with idelalisib and obinutuzumab was used that the disease was stabilized. A new biopsy on 9/2015 showed a CD20 positive tumor, with high degree of proliferation, strong expression of PD-1L and according to the whole transcriptome analysis increased level of PI3K and HR23B, which can be good predictor of response to HDAC inhibitors as valproic acid.
The treatment was changed according to the new findings. He continued with ibrutinib/idelalisib/low dose cyclophosphamide/nivolumab and valproic acid and as of March 2016 the boy is doing very well with Lansky score 100 and OS > 15 months. He has had partial response of the single residual abdominal tumor disease with immune-related adverse His 3rd EFS /7 months/ on personalized therapy is already the longest EFS, compared to 6 months 1st EFS on standard BFM protocol.
The case may illustrate a new variant of Activated PI3K-delta syndrome (APDS). This boy has an atypical germinal mutation in the gene that probably led to the lymphoid hyperplasia, and increases the risk of malignant transformation to B-cell lymphoma. It is our hope that this case illustrates a potential for keeping even children with poor prognosis due to genomically complex cancers at home.

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Pediatric low-grade gliomas with *CRAF* and BRAF gene fusions respond differentially to targeted therapeutics based on their dimerization profiles

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BACKGROUND: Pediatric low-grade gliomas (PLGGs) are the most commonly diagnosed brain tumors in children. PLGGs have been defined by activating BRAF gene mutations/fusions that dysregulate the mitogen-associated protein kinase (MAPK) pathway, leading to

clinical testing of MAPK inhibitors for PLGGs. However, recent large-scale sequencing studies have also identified novel CRAF (or RAF1) fusion proteins, QKI-RAF1 and SRGAP3-RAF1, as potential PLGG driver mutations. As CRAF and BRAF are shared targets of MAPK therapeutics, we sought to investigate the mechanistic and /or differential response of CRAF fusions to clinically relevant RAF inhibitors and downstream pathway inhibitors. We focused on comparing the effects and dependency on RAF dimerization for successful targeting.
MATERIALS & METHODS: Heterologous cell model systems with stable expression of CRAF fusions were generated and used for testing downstream signaling pathways via immunoblotting. Soft agar assays and mouse flank xenografts were used to characterize oncogenic properties. We tested responsiveness to first - and second-generation RAF inhibitors, PLX4720 and PLX8394 respectively, novel RAF dimer inhibitors, MEKi, and mTORi as single agents or in combination. Myc- and Flag-tagged constructs of CRAF fusions were used in co-immunoprecipitation assays to assess dimerization profiles of CRAF fusions with or without inhibitors.
RESULTS: We found that CRAF fusions respond differentially than BRAF-fusions and do not respond to RAF inhibitors, show partial response to single-agent MEK inhibitors, but robustly respond to combinatorial targeting of both MAPK and PI3K pathways and novel RAF dimer inhibitors. Upon comparing the homo- and hetero-dimerization profiles of QKI-RAF1and BRAF fusions in the presence of RAF inhibitors, we found that QKI-RAF1 retains robust homo- and hetero-dimerization that, in contrast, are disrupted in BRAF fusions that respond to RAF inhibitors. This suggests that dimerization is essential for MAPK pathway activation and determines responsiveness to RAF inhibitors. Furthermore, we tested the novel RAF dimer inhibitor, LY3009120, and found that LY3009120 stabilized CRAF fusions in an inactive dimer conformation and suppressed oncogenic potential.
CONCLUSIONS: In summary, our work demonstrates that CRAF fusions are distinct from BRAF fusions in responsiveness to targeted therapies. Our study suggests that molecular classification of PLGGs should inform therapeutic intervention of RAF-altered PLGGs even within RAF-mutant subtypes.

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Translating discovery into cures for children with cancer: Childhood cancer research landscape report

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[1]Children's Cause for Cancer Advocacy, [2]American Cancer Society Cancer Action Network, [3]Children's Hospital of Philadelphia, [4]National Brain Tumor Society, [5]American Academy of Pediatrics, [6]American Childhood Cancer Organization, [7]St. Baldrick's Foundation

Purpose
Cancer is the leading cause of death by disease in the United States for children ages 1 to 19, with more than 14,500 children ages 1 to 19 facing a diagnosis this year. While 5-year survival rates have improved significantly, survivors still experience high rates of long-term and late effects. The goal of this report is to examine the process for – and unique challenges of – conducting childhood cancer research and drug development and how the process is both similar and different than for adults.
Methods
Incidence and mortality data were determined using data from the SEER database. Interviews with key opinion leaders were also conducted. Literature searches provided additional information.
Results:

- Childhood cancers are often biologically different than adult cancers, meaning that childhood-specific research is required.
- Side effects from treatment significantly impact children's health because the treatments occur during a time of vital physical and mental development. Longer survival times mean more time for late effects to appear and further impact health.
- The rarity of childhood cancers makes recruiting children to participate in clinical research challenging and also means that the financial incentives to develop and market drugs specifically for these children are too small to entice significant industry research.

Conclusions and Implications
Childhood cancer has distinct research needs from adult cancer that includes addressing different cancers and a greater focus on reducing side effects. These challenges will require a higher level of coordination between research, advocacy, and regulatory communities than exists in adult cancer drug development.
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