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CTD² PROGRAM HIGHLIGHTS

A Review of the Accomplishments of the CTD² Network

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CONNECTING THE DATA

CTD² in Action: Translating High-Content Genomic Data into New Therapies

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OCG PERSPECTIVE

Building an International Initiative to Infuse Novel Cancer Models into the Research Community

My name is Caitlyn Barrett and I am the Scientific Program Manager for the Human Cancer Model Initiative (HCMI) in the Office of Cancer Genomics (OCG).

PEDIATRIC CANCER RESEARCH

Gabriella Miller Kids First: Exploring Shared Genetic Pathways in Pediatric Diseases

DECONSTRUCTING CANCER

Revealing the Genomic Landscape of Pediatric T-ALL

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CTD² PROGRAM HIGHLIGHTS

A Review of the Accomplishments of the CTD² Network

Subhashini Jagu, Ph.D. and Daniela S. Gerhard, Ph.D.

The Office of Cancer Genomics (OCG) Cancer Target Discovery and Development [2] or CTD^2 initiative was established by the National Cancer Institute (NCI) to accelerate the "translation" of high-throughput, high-content genomic data to the bedside through functional genomics. The CTD^2 initiative is a collaborative network of 13 different research teams, or Centers [3]. The goal of this Network is to develop novel bioinformatics and functional genomics approaches to identify biomarkers, therapeutic targets and perturbagens that affect the targets thereby impeding survival of the cancer cells. The success of the CTD^2 Network has prompted us to take a look back at what the program has accomplished so far.

A major characteristic of the initiative is intra-Network collaboration because there is a continuous communication among the Centers. In addition to conducting individual research projects, the CTD^2 awardees also contributed to trans-network activities, including participation in joint pilot research projects. Most of the trans-Network collaborations are fruitful and are generating high-value publications.

The CTD^2 Network Centers have used multiple platforms to share ideas, data, and resources. All data and tools developed as part of the CTD^2 initiative are made available to the scientific community in a format that can be easily utilized. The examples below demonstrate how CTD^2 broadly shares community resources, confirmation of important scientific concepts, or clinical impact.

Accomplishments

- *Publications:* The CTD^2 Network has been very productive, to date 257 manuscripts [4] have been published and more are submitted. Ten papers have been cited over 100 times.

- *Data Portal:* NCI and CTD^2 Centers developed a Data Portal [5] for deposition of the primary raw/analyzed data. Currently there are 38 diverse datasets in the Data Portal. The Data Portal has an average of 400 unique users per month.

- *CTD^2 Dashboard:* The massive amounts of primary data stored in large databases are often difficult to navigate without bioinformatics expertise and it is also challenging for researchers to determine the significance and reproducibility of a result from the primary data alone. To address this challenge, the Network developed CTD^2 Dashboard which allows easy data

navigation and use by a range of scientists, from computational scientists to cancer experts. The Dashboard gives access to conclusions along with the supporting evidence generated by CTD^2 investigators. To understand more about the Dashboard functionalities, please read the article "CTD^2 Dashboard: A Platform to Explore Evidence-based Observations" [6]. There are currently 132 submissions associated with 45 projects (related submissions are grouped together) in the Dashboard. The CTD^2 Dashboard has over 200 unique users per month.

- *Strength of evidence:* Because of the multiplicity of approaches Network Centers use it is beneficial that the data be presented in a manner that connects results from various approaches to a conclusion. To address this issue, CTD^2 Network developed a multi-Tier framework which defines what constitutes sufficient computational or experimental evidence to support a biologically relevant finding. The Network published a manuscript, "Transforming Big Data into Cancer-Relevant Insight: An Initial, Multi-Tier Approach to Assess Reproducibility and Relevance [7]", which defines the Tiers framework. The multi-Tier framework is being applied to results in the CTD^2 Dashboard.

- *Analytical tools:* To date, the Network has generated nineteen novel algorithms, each with its own function, including the identification of therapeutic targets, gene and molecular networks, driver mutations, and chemical sensitivities. These tools are widely used by hundreds of users each month and can be found at this link [8].

- *CTD^2 supported reagents:* The Network has made available to the community the reagents it has generated, including CRISPR libraries, protein-protein interaction reagents, etc. These reagents foster growth of genomics and precision medicine science. More information on these reagents can be found here [9].

- *Clinical impact:* The CTD^2 Network has been highly successful in generating multiple projects with preclinical and clinical relevance. Many of the hypotheses generated have been tested in mouse models or organoid cultures that represent *in vivo* systems (fulfilling one of the aims articulated in RFA(s)). The results have provided supporting evidence for the initiation of clinical trials: in breast cancer (NCT02066532 [10], NCT02632071 [11]), head and neck cancer (NCT02508246 [12]), and in KRAS mutant non-small cell lung cancer [13] (NCT03095612 [14]).

There are still more challenges to address to enable rapid conversion of molecular data to precision oncology as new data sets and types (cancers from underrepresented minorities, children, data from clinical trials, etc.) are becoming available. Because of the accomplishments, some of which are highlighted above and the collaborative nature of the current Network, the Board of Scientific Advisors approved a new phase of the CTD^2 initiative [15] . The new CTD^2 Network will explore cancer complexity in terms of inter-patient and intra-tumor heterogeneities and their impacts, e.g., on innate or acquired resistance to chemo and immunotherapies. One focus of the new Network will be to take advantage of novel patient-derived cancer

models being developed as part of the Human Cancer Models Initiative (HCMI) [16] for high-throughput functional studies to define biologically relevant targets, modulators and biomarkers.

In summary, the Network strives to integrate large-scale genomic data with systems biology analyses and high-throughput molecular modulation (small-molecules/CRISPR/RNAi) of the cancer phenotype. The Network accelerates the discovery process by sharing knowledge and resources. The Network has made great accomplishments through collaborations and looks forward to making more discoveries by ensuring that the new Network analyzes the data expeditiously and integrates into the body of knowledge the initiative has already amassed.

Back to Top

CONNECTING THE DATA

CTD² in Action: Translating High-Content Genomic Data into New Therapies

Michael Roth, Ph.D.

Large-scale molecular analyses have provided an unprecedented global view of the molecular defects in cancers and promise to revolutionize precision cancer medicine by guiding the development of therapies that are matched to genomic alterations in tumors. Cancer is a heterogeneous disease which explains why there are varying responses to therapy. This heterogeneity poses a daunting challenge for clinicians managing a patient's disease. The Cancer Target Discovery and Development [2] (CTD^2) Center at The University of Texas Southwestern (UTSW) is using a systematic approach to identify novel vulnerabilities linked to driver mutations in the etiology of non-small cell lung cancer (NSCLC).
The multiple oncogenotypes that cause lung cancer are known to confer different responses to currently approved therapeutics. One of of the most common drivers in cancers is the RAS gene family. Thus far attempts to find inhibitory drugs for KRAS have been unsuccessful. The CTD^2 center at UTSW has used small interfering RNA (siRNA) libraries, targeting ~18,000 human genes, to identify essential genes for the survival of NSCLC cell lines that express mutant KRAS protein. The screening helped to identify a gene set, significantly enriched with in the KRAS-mutant cohort, which encoded machinery responsible for exporting proteins from the nucleus. Among these genes was XPO1, which encodes a nuclear export receptor for which an FDA approved inhibitor exists.

Proving the druggable target XPO1 is essential to KRAS mutant NSCLC cells
A series of loss-of-function experiments were performed in 55 NSCLC cell lines and a strong correlation was observed between toxicity and the presence of an oncogenic KRAS mutation. UTSW researchers tested two XPO1 inhibitors on NSCLC cell lines, either expressing normal or mutant KRAS, for their effects on cell growth. The KRAS mutant cell lines were significantly sensitive to the XPO1 inhibitors. The sensitivity to XPO1 inhibitors was completely lost when KRAS mutant cell lines were engineered to have a drug resistant mutation in XPO1. This proved that the only effect of the XPO1 inhibitors on the sensitive cell was through binding to XPO1.

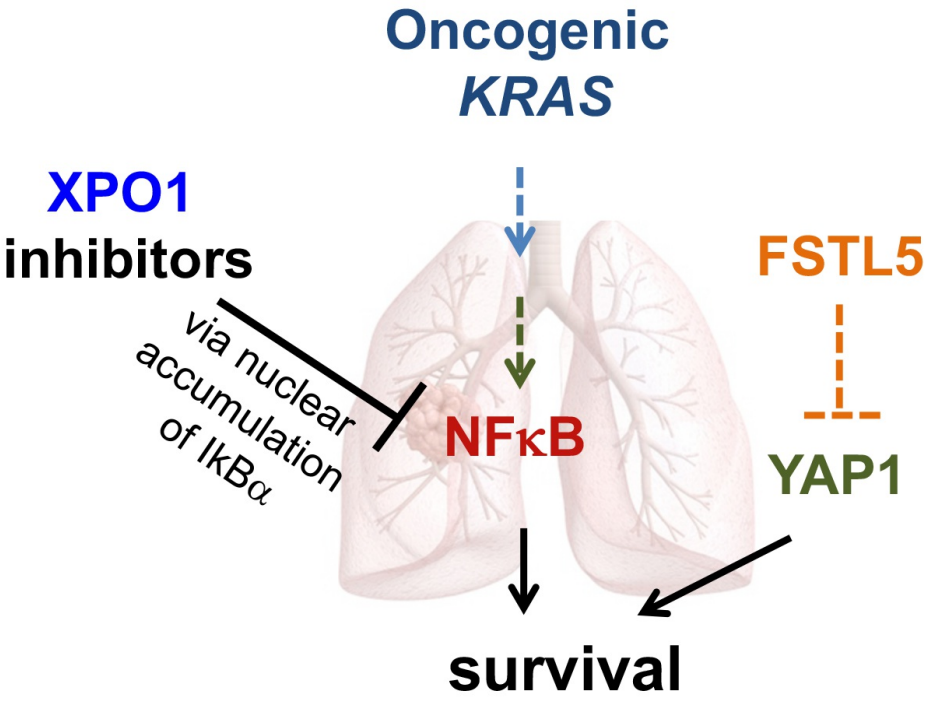

Figure1: XPO1 inhibitors acting NF-kappa B pathwat in KRAS driven lung cancer

Exceptions to the rule define the mechanisms of resistance to XPO1 inhibitors and identify ways to defeat it

A major obstacle for all cancer therapies is the eventual development of drug-resistant and recurring tumors. UTSW researchers observed a subset of NSCLC cell lines expressing oncogenic KRAS mutations were not sensitive to XPO1 inhibitors. These cell lines contained mutations in the FSTL5 gene. Mutations in the FSTL5 were identified in 10% of lung adenocarcinomas in the TCGA database. To directly test the role of FSTL5 in resistance to XPO1 inhibitors, they depleted FSTL5 by siRNA and found that it reduced sensitivity of KRAS-mutant cell lines to XPO1 inhibitors.

Previously it was reported that Yap signaling produced resistance to loss of KRAS signaling in mouse lung and pancreatic cancers. UTSW scientist found that human lung adenocarcinomas with FSTL5 mutations had significant increases in YAP1 protein expression. To test this directly, they depleted FSTL5 by siRNA in NSCLC cell lines and observed an increase in YAP1 protein. Over-expression of YAP1 in NSCLC cell lines driven by oncogenic mutant KRAS induced resistance to XPO1 inhibitors. Inhibiting YAP1 transcription factor activity either chemically or with siRNA restored sensitivity of KRAS mutant cell lines to XPO1 inhibitors. Thus, a way to prevent resistance to XPO1 inhibition is to treat tumors with inhibitors of YAP1.

A clinical trial

The experimental findings of researchers at UTSW have provided a fast-track for the discovery of a novel therapy for KRAS driven NSCLC and have identified a potential approach for preventing resistance to XPO1 inhibition. Scientists at pharmaceutical company Karyopharm have independently replicated the results of the UTSW researchers. Based upon these results, Karyopharm has signed a contract with oncologists at UTSW to begin a clinical trial to determine if the XPO1 inhibitor is a good therapeutic choice for a subset of NSCLC driven by oncogenic KRAS mutations. This is an example of the goals of the CTD^2, to accelerate the translation of discoveries into new cancer therapies by bridging the gap between cancer genomics and precision oncology.

For further reading of the original XPO1 article click the link below.
https://www.ncbi.nlm.nih.gov/pubmed/2768070 [17]

Back to Top

OCG PERSPECTIVE

Building an International Initiative to Infuse Novel Cancer Models into the Research Community

Caitlyn Barrett, Ph.D.

Caitlyn Barrett, Ph.D.
Program Manager, Human Cancer Model Initiative

My name is Caitlyn Barrett and I am the Scientific Program Manager for the Human Cancer Model Initiative (HCMI) in the Office of Cancer Genomics (OCG). In my role within the HCMI, I am helping to establish communication pathways and build the foundation for collaboration that will enable the completion of the Initiative's aim to develop as many as 1000 next-generation cancer models, established from patient tumors and accompanied by clinical and molecular data. In order to accomplish this lofty goal, the NCI has funded two Cancer Model Development Centers (CMDCs) and formed an international collaboration with Cancer Research UK, the foundation Hubrecht Organoid Technology, and the Wellcome Trust Sanger Institute. I anticipate that the cancer models created by this international consortium will enable researchers to advance cancer research and precision medicine, connecting *in vitro* findings to clinical biology.

I began my position with the OCG in September 2016, following eight years of bench research in Cell Biology. While obtaining my Doctoral degree in Cancer Biology, I was introduced to the revolutionary primary cell culture method developed in the lab of Dr. Hans Clevers, organoids. Upon graduation, I moved into a postdoctoral position studying the pathways involved in the premature death of dopaminergic neurons in Parkinson's Disease. My goal in following such disparate research paths was to garner a perspective on the complex machinery that is subverted to allow cells to live prolonged and malignant lives (as in cancer) or leads to the premature death of cells (as seen in neurodegeneration). Throughout my research experience, I always returned to the idea that the culturing of primary cells from patients with diseases such as cancer would provide indispensable research tools.

Fortunately for me, leaders within the National Cancer Institute (NCI) had also recognized the value of next-generation culturing and were working to initiate a program that would create organoid and other next-generation cancer models from patient tissue. This technology would allow for the production of cancer models that accurately recapitulate human tumors. The NCI, Hubrecht Organoid Technology, Wellcome Trust Sanger Institute, and Cancer Research UK developed a collaboration to produce these next-generation cancer models. I joined the OCG because the HCMI enabled me to contribute to a program that would develop cancer models as tools for the research community, a goal I had cultivated during my time as a research scientist. When I began my position, contracts for the NCI's CMDCs were being awarded. The CMDCs are funded by the NCI and tasked with producing next-generation cancer models from clinical samples. One of my first responsibilities within the HCMI is to work with the CMDCs and other collaborators within the NCI to develop methods for tracking model development and design a searchable database that will enable end-users to identify next-generation cancer models applicable to their research. Completion of these tasks will ensure that the models are easily accessible to the research community in a timely manner.

Why next-generation cancer models? Having worked in a Cancer Biology lab, I am aware of the limitations of current cancer cell lines as accurate cancer models. Most cancer cell lines that are used in research labs today were established decades ago. These traditional cancer cell culture models have been useful for *in vitro* experiments to understand cancer biology; however, cancer cell lines do not recapitulate all aspects of the disease and often lack the complexity and architecture of human tumors. Furthermore, cell lines lack information about

the original tumors and patient from which they were derived, posing a problem for scientists aiming to couple *in vitro* findings with clinical biology. Next-generation culturing techniques, including conditionally reprogrammed cells (CRCs) and organoids, offer distinct advantages over current cancer cell lines. Because they are not clonally-derived, they better represent the heterogeneity of the original tumor. They are genotypically and phenotypically stable in culture over time, retaining characteristics of the original tumor, and they do not need to be immortalized through methods that alter their genetic make-up. The HCMI is further enhancing the benefit of the models they produce by providing pertinent patient clinical and molecular characterization data for each model. This means that scientists that use these models will know about the genetic mutations within the original tumor and model as well as the genetic makeup of the normal tissue of the patients. Researchers will also have insight into the treatments that patients have undergone and will be able to choose models based on driver mutations in which they are interested. What's more, as these models are more accurate representations of the original tumor and amenable to high-throughput screening, they will be important tools to enhance precision medicine.

As the HCMI project has only recently begun, there is a great deal to be done up-front to ensure program success. Following a fruitful Kickoff Meeting in January, 2017, a major goal of the Initiative is to establish processes to work together, across nations, in a seamless fashion. Important tasks that I am working with the OCG director Daniela Gerhard and members of the HCMI to complete include: (1) establishing mechanisms for effectively and securely sharing information amongst the members of the Initiative, (2) monitoring and aiding in the development of standard operating procedures for all aspects of sample collection and model development, and (3) ensuring that databases and the distributor are prepared to receive data and models so that subsequent distribution to the research community is efficient. Throughout this process, I am learning a great deal about working within the government and across country lines. Working within an international consortium has taught me that molecular and patient data sharing is a highly regulated process and that each organization and country has protocols for how data is distributed. The challenge that I am currently working to resolve is determining how to harmonize data so that it translates effectively between countries and make it accessible to end-users from around the world. I have also learned that, depending on the clinical center gathering patient-pertinent information, informed consent can vary greatly, especially as it pertains to how much information about the study is available to the patient and his/her doctor. While this document can vary from place to place, each informed consent is set up to ensure that patients are protected while involved in the study. Our goal has been to develop informed consent that maximizes patient protection while still giving the HCMI the freedom to create and distribute the models for the utmost benefit to the research community and, ultimately, the patient population. Importantly, I am also becoming accustomed to working within large groups where each member possesses an essential skill which must be combined with those of other contributors to complete a large project. For instance, consortium management, scientists, programmers, and web developers are all essential contributors to the design and implementation of model development tracking databases and searchable databases intended to benefit end-users. The execution of the HCMI is a huge team effort that involves the input of all contributors and necessitates communication, organization, and persistence, skills that I developed as a research scientist but will be finely honed in my new position within the HCMI.

My ultimate aspiration is that I become the liaison which consortium and CMDC members, contributors to our resources, and end-users within the scientific community can approach for any questions or needs that they have in relation to the HCMI. My role is as a facilitator within the HCMI and my purpose is to ensure the success of the program. I intend, as the program begins and continues to meet its goals and distribute models to the scientific community, to address any needs that arise amongst our collaborators within the program as well as researchers using our cancer models. To guarantee accessibility of the models and data and ensure that a user-friendly interface is always forefront when it comes to data and model access are tasks that I think are essential to the success of the HCMI and will be my primary mission as program manager.

The HCMI, through development of novel cancer models as well as sharing of successes and failures in model development with the research community, will provide an invaluable resource to researchers. I am thrilled to be

contributing to the HCMI's goal to provide patient-relevant models and related clinical and molecular data to researchers as a community resource in an effort to advance cancer research and more fully understand how *in vitro* findings are related to clinical biology. Here, I am recognizing my personal goal to contribute to the progress of cancer research by assisting in the development of patient-derived cancer models that will revolutionize the way that laboratory research can be translated to the clinic.

Back to Top

PEDIATRIC CANCER RESEARCH

Gabriella Miller Kids First: Exploring Shared Genetic Pathways in Pediatric Diseases

Jaime Guidry-Auvil, Ph.D. and Freddie Pruitt, Ph.D

In the United States an estimated 10,380 children under the age of 15 will be diagnosed with cancer, and an estimated 1,250 children die from their disease (www.cancer.gov [18]). Childhood cancer is the leading cause of death by disease in the US from just past the age of infancy through young adulthood, yet there continues to be a general lack of "kid-specific" treatment options for these young patients. The NCI Office of Cancer Genomics (OCG) has been committed to advancing the understanding of the biology, as well as improving therapeutic mortality and morbidity, of childhood cancers through its highly-successful "Therapeutically Applicable Research to Generate Effective Treatments [19]" (TARGET) initiative and others. There have been large improvements in therapeutic outcomes over the past few decades for pediatric cancer patients generally. However, there remain multiple cancer subtypes with low cure rates and slow progression in the development of novel effective treatments.

While cancer is largely responsible for pediatric mortality by disease, birth defects are the leading cause of death during the first year of life. Of the approximate four million children born each year in the US, 1 in every 33 infants will have a birth defect. These pediatric conditions have profound, lifelong effects on patients and their families regardless of whether the children affected survive or succumb to their disorders. The fields of pediatric oncology and developmental biology have made major discoveries to advance our understanding of disease and reduce the number of childhood mortalities, but more must be learned to facilitate the development of new diagnostics, treatments, and cures for these children.

The Gabriella Miller Kids First Pediatric Research Program [20] (GMKF, Kids First) is a trans-NIH initiative devoted to exploring and analyzing genetic predisposition and/or somatic association within various childhood cancers and structural birth defects. The program developed from the dedication of a young patient who wanted to make a difference for science and medicine. At just nine years of age, Gabriella Miller, for whom the Kids First initiative

was named, was diagnosed with a rare tumor affecting the pons portion of the brainstem called diffuse intrinsic pontine glioma [21] (DIPG). This aggressive and inoperable form of brain cancer is highly resistant to chemotherapy and paralyzes the nervous system of those patients. In effort to further progress and discovery of effective treatments for other children suffering with pediatric disease, 10-year-old Gabriella petitioned Congress for increased support for pediatric research before her untimely passing in 2013. In 2014, the Gabriella Miller Kids First Research Act [22] was signed into law, authorizing $12.6 million each year for 10 years to support pediatric research within the NIH.

The goals of the Gabriella Miller Kids First Pediatric Research Program include generating comprehensive genomic characterization across pediatric cohorts with a known or suspected genetic role, or somatic role in tumors. To that end, whole genome sequencing of patient tissues, along with samples from potentially affected and non-affected relatives, is providing rich data for investigators to analyze. Deep whole exome and transcriptome sequencing are offered when matched patient tumors are available. Representatives from OCG and NCI's Clinical Therapy Evaluation Program [23] (CTEP) are working with colleagues from other NIH Institutes to provide guidance and expertise to the Kids First program and facilitate the collection of sequence and clinical data from existing patient cohorts that include a familial component. To study cancer susceptibility, cohorts selected for study under this program include acute lymphoblastic leukemia; Hodgkin's and non-Hodgkin's lymphomas; Ewing's sarcoma (germline parent-child trios only); treatment-resistant pediatric osteosarcoma; neuroblastoma; and a subset of various central nervous system (CNS) and non-CNS solid tumors from the BASIC3 study. To better understand the etiology of birth defects, sequence data is now in the pipeline for patient cohorts with congenital heart defects; congenital diaphragmatic hernia; orofacial clefts; syndromic cranial dysinnervation disorders; disorders of sex development; congenital hearing loss; and, adolescent idiopathic scoliosis. The genomic data from cohorts sequenced to date will be made available to the research community through NIH databases including NCBI's database of Genotypes and Phenotypes [24] (dbGaP) and Sequence Read Archive [25] (SRA), as well as NCI's Genomic Data Commons [26] (GDC).

The pediatric research community benefits from access to well-curated medical, genomic, and clinical data from large numbers of children, as has been provided by programs like TARGET [19], particularly when available alongside the computational resources to analyze it. Providing researchers access to robust and complete data sets enables them to answer new questions, explore different lines of research, and more efficiently conduct large-scale analyses, potentially leading to the development of novel therapeutic interventions in children with cancer or structural birth defects. As such, the GMKF program is in the process of building a "Pediatric Data Resource [27]" that will serve as a centralized database or portal to access and analyze curated clinical and genomic data encompassing childhood cancers and structural birth defects from large numbers of patients and their immediate family members. This database will provide a searchable resource for all investigators to mine data for the purposes of identifying genetic or somatic pathways that underlie pediatric cancer and structural birth defect etiology and to further explore whether shared genetic pathways exist between childhood cancer and structural birth defects.

Facilitating these types of dual analyses will hopefully accelerate research across the pediatric disease spectrum towards more effective and innovative diagnostic and curative therapies in children. The Kids First Pediatric Data Resource will begin development in late 2017, and available to researchers across the entire biomedical research community once established. The initiative is intended to serve as a platform for investigators to study gene pathways involved in organ formation and normal cell growth across disease conditions and for new collaborations between clinical and basic researchers at all career levels.

To find out more about the NIH Gabriella Miller Kids First Pediatric Research Program, please click here [20].

Back to Top

DECONSTRUCTING CANCER

Revealing the Genomic Landscape of Pediatric T-ALL

Stephen Hunger, M.D. and Charles Mullighan, MBBS, MSc, M.D.

T-lineage acute lymphoblastic leukemia (T-ALL) comprises 15-20% of childhood ALL and has historically been associated with inferior outcome to B-cell ALL (B-ALL). Recent studies have used genome-wide sequencing approaches to identify new subtypes and targets of mutation in B-ALL, but comprehensive sequencing studies of large cohorts of T-ALL have not been performed. A collaborative team from the Children's Oncology Group (COG), the NCI TARGET initiative [19] and St Jude Children's Research Hospital report results of an integrated genomic analysis of childhood and young adult T-ALL in Nature Genetics [28].

The research team sought to gain a comprehensive understanding of the landscape of genomic alterations present in childhood T-ALL, to identify constellations of mutations and associations of mutations with T-ALL subtype, clinical features and outcome. A particular goal was to identify genetic alterations, for example in signaling pathways, that might be "actionable" by treatment with available drugs. To accomplish this, the group studied 264 children with T-ALL treated on the COG AALL0434 study of combination chemotherapy in T-ALL and lymphoblastic lymphoma (NCT00408005 [29]) with available stored leukemic cells obtained from bone marrow or peripheral blood at diagnosis, and a matched sample obtained at remission to distinguish somatic (tumor-acquired) genetic alterations from inherited genetic variations. The samples were subjected to whole exome sequencing to identify sequence mutations, and transcriptome sequencing to classify T-ALL samples by gene expression profiling and detect rearrangements resulting in promoter hijacking or that create novel fusion proteins. Single nucleotide polymorphism array analysis was used to detect DNA copy number alterations. Multiple *in silico* analysis algorithms and mutant allele expression were used to infer "driver" status to mutated cancer genes. Overall, 2694 genes were found to be mutated, and 106 of these were considered cancer drivers. This analysis illustrated the issue of the "long tail" of mutations in cancer genomic analyses, in that 24 genes harboring presumed pathogenic mutations were mutated in only a single case. An unexpected finding was that half of the 106 driver genes had not previously been identified in pediatric T-ALL, or in any prior studies of T-ALL. Many of the mutations identified were subclonal, including those in well-characterized oncogenic drivers such as NOTCH1, indicating that these mutations are acquired as secondary events following leukemia-initiating lesions (typically chromosomal rearrangements deregulating T cell transcription factors). While previously recognized, the extent of subclonal mutation was more extensive than previously appreciated: approximately one third of cases had multiple subclonal NOTCH1 mutations.

Multiple modalities of genomic data were used to assign the majority of T-ALL cases to groups defined by alteration and overexpression of T cell transcription factor genes such as *TAL1, TLX1,* and *TLX3.* This had two important implications: first, this detailed genomic analysis identified multiple new partners of rearrangement of many of these genes, and the second showed the genomic approaches used could not identify the mechanism of deregulation in all cases. To further pursue this, whole genome sequencing was performed for 25 cases, which identified chromosomal rearrangements in several, but not all cases. Key reasons for this discrepancy is that not all rearrangements encode a chimeric fusion transcript detectable by transcriptome sequencing (e.g. where rearrangement breakpoints lie outside of genes) or because deregulation is due to focal non-coding alterations.

Integrated analysis identified 10 recurrently mutated pathways in T-ALL: transcriptional regulation, cell cycle regulation and tumor suppression, NOTCH1 signaling, epigenomic regulation, PI3K-AKT signaling, JAK-STAT signaling, Ras signaling, ribosomal function, ubiquitination and RNA processing. The nature of alterations in each

pathway, and the cumulative frequency of mutation of each pathway varied significantly according to T-ALL subtype, with multiple striking subtype-specific associations identified – for example mutations of the deubiquitinating gene *USP7* in *TAL1*-rearranged ALL. In a complementary analysis, researchers examined 30 most frequently mutated genes that exhibit associations with T-ALL subtype. Each T-ALL subtype is considered to originate from different stages of normal thymic T cell development, and this analysis showed several striking associations between mutated genes and developmental stage – a notable example being signaling pathways, with NRAS/FLT3 mutations in immature T-ALL, JAK-STAT mutations in HOXA1 cases, PTPN2 in TLX1 cases and PI3K cases in TAL1-rearranged cases. This suggests not only that these mutations have key oncogenic roles in each of these subtypes, but that different kinase signaling pathways have roles in different developmental stages of T cell development.

The frequency of mutation of signaling pathways was higher than previously recognized in T-ALL, with relative mutual exclusivity of involvement of the major pathways (JAK-STAT, PI3K-AKT and Ras) in addition to the subtype-specific alterations described above. FDA-approved agents targeting each pathway are in clinical use, such as ruxolitinib and multiple PI3K/AKT and MEK inhibitors, creating opportunities to test precision oncology approaches in pediatric and young adult T-ALL. An additional finding from this analysis was of multiple signaling mutations, either of the same or different pathways, in many cases. This is of clinical significance as it raises the possibility that if such mutations reside in different subclones, targeted therapy against singular mutations/pathways may suppress or eliminate one clone, but allow another to proliferate unchecked. The research team examined the mutant allele fraction (MAF) of the mutations with the highest and next highest MAF in a subset of cases with JAK mutations. The MAF levels remained consistent with mutations observed within an individual clone for a majority of cases expressing JAK mutations, even when the case showed expression of multiple signaling mutations between clones. These findings support a rational for using combination pathway inhibitors towards preventing subclonal selection rather than using single agent therapies. An additional finding was the high frequency of mutations in genes encoding epigenetic regulators (68% of the cohort). While these mutations affected a diverse range of mechanisms of regulation, many are being pursued as therapeutic targets, suggesting that these agents may hold promise for a substantial proportion of T-ALL cases.

Given the historically poor outcomes for T-ALL, a key goal of the study was to examine associations between genetic alterations – either at the gene or pathway level – with outcomes such as poor initial response to therapy, as measured by levels of minimal residual disease at the completing of remission-induction therapy, and event-free and overall survival. Multiple prior studies have examined small numbers of genes, or historic cohorts, with conflicting results. In the Nature Genetics study, relatively few significant associations with outcome were found. This is likely to be due to the gratifying low relapse rate of only 7.5% in this cohort, reflective of significant recent improvement in survival in COG T-ALL studies. In addition, a small number of cases with very poor outcome and failure of adequate response to initial therapy were not studied because there was no suitable remission or germline material available. Thus, future studies focused on the genomic basis of treatment failure in such cases will be of interest.

The study has provided a genomic T-ALL “dictionary” that will be of great interest to researchers and clinicians studying and treating patients with this form of leukemia. The thorough description of the constellations of mutations that define each subtype will enable the development of new generation of experimental models that faithfully recapitulate human disease. The findings also provide an essential resource for interpretation of results of diagnostic genome sequencing studies that are rapidly entering clinical practice. All data are currently available through the TARGET data matrix (https://ocg.cancer.gov/programs/target/data-matrix [30]) and will be available in the Genomic Data Commons shortly. Results of the study may be explored and manipulated interactively at the St Jude Pediatric Cancer genomics website (PeCan: https://pecan.stjude.org/proteinpaint/study/target-tall) [31].

Back to Top

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Links
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