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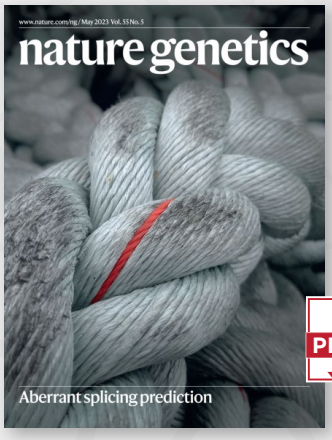
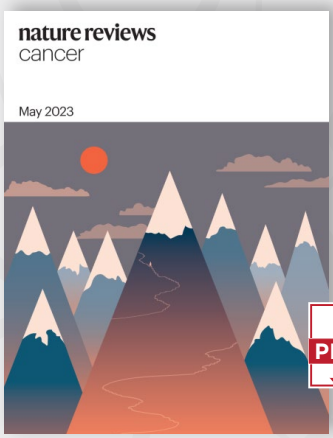
A Selective Survey of DarwinHealth's Published Contributions to the Global Scientific and Clinical Literature Focused on Cancer Therapeutics

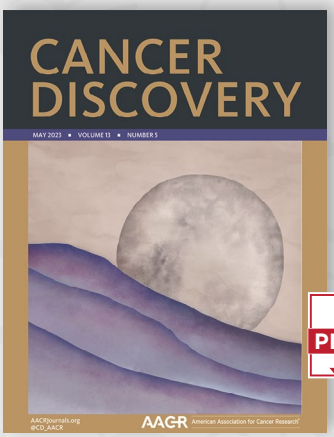


A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors

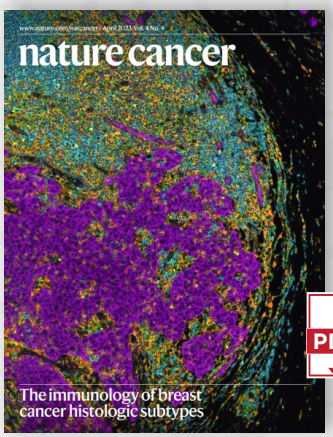
Oral Selinexor–Dexamethasone for Triple-Class Refractory Multiple Myeloma



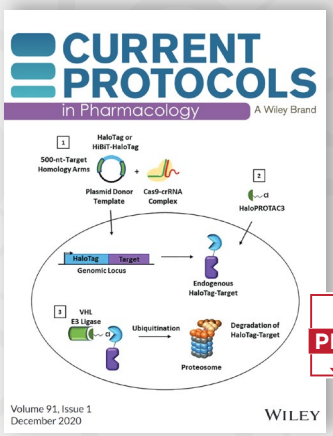
The recurrent architecture of tumour initiation, progression and drug sensitivity



A Transcriptome-Based Precision Oncology Platform for Patient–Therapy Alignment in a Diverse Set of Treatment-Resistant Malignancies

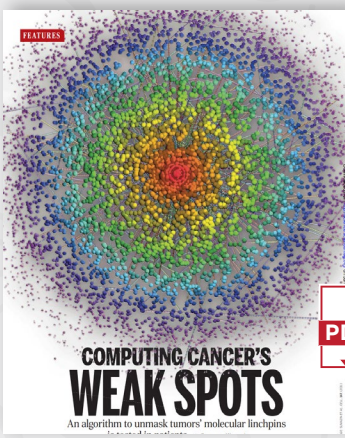


Network-based assessment of HDAC6 activity predicts preclinical and clinical responses to the HDAC6 inhibitor ricolinostat in breast cancer



A Patient-to-Model-to-Patient (PMP) Cancer Drug Discovery Protocol for Identifying and Validating Therapeutic Agents Targeting Tumor Regulatory Architecture

Fimepinostat (CUDC-907) in patients with relapsed/refractory diffuse large B cell and high-grade B-cell lymphoma: report of a phase 2 trial and exploratory biomarker analyses



COMPUTING CANCER'S WEAK SPOTS
An algorithm to unmask tumors' molecular linchpins is tested in patients

Systematic elucidation and pharmacological targeting of tumor-infiltrating regulatory T cell master regulators

nature genetics

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Clinical and Translational Impact and Applications

This seminal paper describes the **DarwinOncoTreat** methodology to predict drug sensitivity on an individual patient/tumor basis. This is accomplished by using the **VIPER** algorithm to assess the differential activity of the Master Regulator proteins of a specific tumor in drug vs. vehicle-control-treated cell lines chosen to optimally recapitulate patient-specific MR activity.

A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors

Mariano J. Alvarez[1,2,40], Prem S. Subramaniam[1,40], Laura H. Tang[3], Adina Grunn[1], Mahalaxmi Aburi[1], Gabrielle Rieckhof[4], Elena V. Komissarova[1], Elizabeth A. Hagan[5], Lisa Bodei[3,6], Paul A. Clemons[7], Filemon S. Dela Cruz[8], Deepti Dhall[9], Daniel Diolaiti[8], Douglas A. Fraker[10], Afshin Ghavami[11], Daniel Kaemmerer[12], Charles Karan[13], Mark Kidd[14], Kyoung M. Kim[15], Hee C. Kim[15], Lakshmi P. Kunju[16,17,18], Ülo Langel[19,20], Zhong Li[21], Jeeyun Lee[15], Hai Li[13], Virginia LiVolsi[10], Roswitha Pfragner[22], Allison R. Rainey[8], Ronald B. Realubit[13], Helen Remotti[23], Jakob Regberg[19], Robert Roses[10], Anil Rustgi[10], Antonia R. Sepulveda[23], Stefano Serra[24], Chanjuan Shi[25], Xiaopu Yuan[9], Massimo Barberis[6], Roberto Bergamaschi[26], Arul M. Chinnaiyan[16,17,18,27,28], Tony Detre[21], Shereen Ezzat[24], Andrea Frilling[29], Merten Hommann[12], Dirk Jaeger[30], Michelle K. Kim[31], Beatrice S. Knudsen[9], Andrew L. Kung[8], Emer Leahy[11], David C. Metz[10], Jeffrey W. Milsom[32], Young S. Park[15], Diane Reidy-Lagunes[3], Stuart Schreiber[7,33], Kay Washington[25], Bertram Wiedenmann[34], Irvin Modlin[35]* and Andrea Califano[1,36,37,38,39]*

We introduce and validate a new precision oncology framework for the systematic prioritization of drugs targeting mechanistic tumor dependencies in individual patients. Compounds are prioritized on the basis of their ability to invert the concerted activity of master regulator proteins that mechanistically regulate tumor cell state, as assessed from systematic drug perturbation assays. We validated the approach on a cohort of 212 gastroenteropancreatic neuroendocrine tumors (GEP-NETs), a rare malignancy originating in the pancreas and gastrointestinal tract. The analysis identified several master regulator proteins, including key regulators of neuroendocrine lineage progenitor state and immunoevasion, whose role as critical tumor dependencies was experimentally confirmed. Transcriptome analysis of GEP-NET-derived cells, perturbed with a library of 107 compounds, identified the HDAC class I inhibitor entinostat as a potent inhibitor of master regulator activity for 42% of metastatic GEP-NET patients, abrogating tumor growth in vivo. This approach may thus complement current efforts in precision oncology.

Emerging efforts in precision oncology are largely predicated on the identification of 'actionable' oncogene mutations, whose pharmacological inhibition elicits oncogene addiction[1]. Despite initial successes and clinical deployment of this concept, several limitations have emerged[2]. First, multiple studies[3] have shown that most adult malignancies lack actionable mutations or harbor mutations either in non-druggable oncogenes (for example, *RAS and MYC* family proteins) or in genes of poorly characterized therapeutic value[4]. Moreover, while mutation-directed therapy often achieves a remarkable initial response, this is almost inevitably followed by relapse and emergence of drug resistance[5,6]. Finally, analysis of hundreds of cell lines and compounds shows that, with some exceptions—such as for *BRAF*, *ERBB2*, *FGFR* and *ALK1* inhibitors—mutations are poor predictors of drug sensitivity[7]. This is not entirely surprising, as drug sensitivity is a complex (dynamic, multifactorial, polygenic) phenotype. As such, there is urgent need for novel approaches that complement and extend oncogene addiction.

Recent results on the aberrant regulatory logic of cancer-related phenotypes have highlighted the existence of master regulator proteins, whose coordinated activity within tightly regulated modules (tumor checkpoints) is strictly necessary for tumor state initiation and maintenance[8]. Consistently, as shown in leukemia[9], lymphoma[10,11], glioblastoma[12], prostate[13,14], neuroblastoma[15] and breast cancer[16], genetic or pharmacological inhibition of master regulator proteins leads to tumor-checkpoint collapse and loss of tumor viability. Indeed, master regulators are highly enriched in essential[10] and synthetic-lethal[11–15,16] proteins, thus representing a novel class of non-oncogene dependencies[17,18] and pharmacological targets. Their mechanistic role in tumor cell state maintenance results from their mechanistic transcriptional control of gene expression signatures (GES) representing the tumor cell's transcriptional identity. Master regulator proteins can be efficiently and systematically elucidated using the MARINa (Master Regulator Inference algorithm)[12,19] and VIPER (Virtual Proteomics by Enriched Regulon analysis)[20] algorithms—the latter allowing analysis on an individual sample basis, a prerequisite for precision oncology applications. These algorithms were extensively validated[11–13,16,21].

Thus, the rationale for this methodology (OncoTreat) is that small-molecule compounds capable of inducing tumor-checkpoint

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Author contributions

A.C., I.M. and M.J.A. conceived the study and wrote the manuscript. A.C. and I.M. assembled and coordinated the consortium activities. M.J.A. and A.C. conceptualized and designed the algorithms and the experiments; M.J.A. developed the algorithms and analyzed the data. P.S.S. designed and performed the experimental assays, analyzed the resulting data and wrote the manuscript. L.H.T. assessed GEP-NET sample quality, tumor purity and tumor pathology. A. Grunn and E.V.K. performed sample preparation, RNA isolation and immunohistochemistry assays, and managed the sample repository; T.D., G.R., M.A., E.A.H. and Z.L. coordinated the study logistics and sample procurement across participating institutions; P.A.C. and S. Schreiber conceived and performed the differential drug response curve assays and analyzed the data; C.K., R.B.R. and H.L. performed the RNA-Seq profiling following drug perturbation assays. F.S.D.C., D. Diolaiti, A.R.R. and A.L.K. performed in vivo experiments and analyzed the data; R.P., I.M. and M.K. contributed GEP-NET-derived cell lines; L.B., D. Dhall, D.A.F., A. Ghavami, D.K., M.K., K.M.K, H.C.K., L.P.K., U.L., J.L., V.L.V., H.R., J.R., R.R., A.R., A.R.S., S. Serra, C.S., X.Y., M.B., R.B., A.M.C., S.E., A.F., M.H., D.J., M.K.K., B.S.K., E.L., D.C.M., J.W.M., Y.S.P., D.R.-L., K.W. and B.W. contributed fresh-frozen GEP-NET samples.

Competing interests

M.J.A. is Chief Scientific Officer and equity holder at DarwinHealth, Inc., a company that has licensed some of the algorithms used in this manuscript from Columbia University. A.C. is founder and equity holder of DarwinHealth Inc. Columbia University is also an equity holder in DarwinHealth Inc.

Additional information

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[1]Department of Systems Biology, Columbia University, New York, NY, USA. [2]DarwinHealth Inc, New York, NY, USA. [3]Memorial Sloan Kettering Cancer Center, New York, NY, USA. [4]Institute for Systems Genetics, New York University Langone Medical Center, New York, NY, USA. [5]Department of Urology, Columbia University, New York, NY, USA. [6]Division of Pathology, European Institute of Oncology, Milan, Italy. [7]Broad Institute of Harvard and MIT, Cambridge, MA, USA. [8]Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [9]Cedars-Sinai Medical Center, Los Angeles, CA, USA. [10]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [11]PsychoGenics Inc., Tarrytown, NY, USA. [12]Department of General and Visceral Surgery, Zentralklinik, Bad Berka, Germany. [13]Sulzberger Columbia Genome Center, Columbia University, New York, NY, USA. [14]Wren Laboratories, Branford, CT, USA. [15]Division of Hematology Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea. [16]Michigan Center for Translational Pathology, University of Michigan Medical School, Ann Arbor, MI, USA. [17]Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA. [18]Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI, USA. [19]Department of Neurochemistry, the Arrhenius Laboratories for Nat. Sci., Stockholm University, Stockholm, Sweden. [20]Laboratory of Molecular Biotechnology, Institute of Technology, University of Tartu, Tartu, Estonia. [21]Falconwood Foundation, New York, NY, USA. [22]Institute of Pathophysiology and Immunology, Medical University of Graz, Graz, Austria. [23]Department of Pathology, Columbia University, New York, NY, USA. [24]Department of Pathology, University Health Network, University of Toronto, Toronto, Canada. [25]Department of Pathology, Vanderbilt University Medical Center, Nashville, TN, USA. [26]Division of Colon and Rectal Surgery, State University of New York, Stony Brook, NY, USA. [27]Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, MI, USA. [28]Department of Urology, University of Michigan Medical School, Ann Arbor, MI, USA. [29]Imperial College London, London, UK. [30]Medical Oncology, National Center for Tumor Diseases Heidelberg, University Medical Center Heidelberg, Heidelberg, Germany. [31]Mount Sinai School of Medicine, New York, NY, USA. [32]Department of Surgery, New York-Presbyterian Hospital, Weill Cornell Medicine, New York, NY, USA. [33]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA. [34]Department of Internal Medicine, Division of Gastroenterology, Charite, Universitätsmedizin Berlin, Berlin, Germany. [35]Emeritus Professor Gastrointestinal Surgery, School of Medicine, Yale University, New Haven, Connecticut, USA. [36]Department of Biomedical Informatics, Columbia University, New York, NY, USA. [37]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA. [38]J.P. Sulzberger Columbia Genome Center, Columbia University, New York, NY, USA. [39]Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY, USA. [40]These authors contributed equally: Mariano J. Alvarez, Prem S. Subramaniam. *e-mail: imodlin@irvinmodlin.com; califano@cumc.columbia.edu

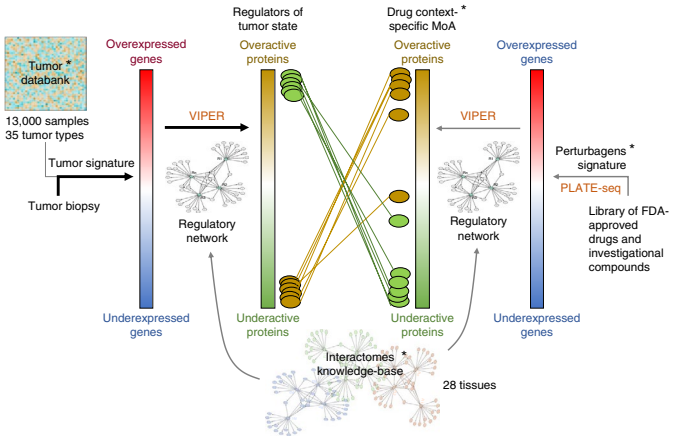

Fig. 6 | Schematic diagram for the OncoTreat clinical pipeline. The pipeline consists of a series of pre-computed (*) components, including a reference set of more than 13,000 tumor expression profiles representing 35 different tumor types, a collection of 28 tissue context-specific interactomes and a database of context-specific MoA for >400 Food and Drug Administration (FDA)-approved drugs and investigational compounds in oncology. This database is obtained by perturbing at least two cell lines per tissue type—which, in a quasi-orthogonal fashion, recapitulate the top master regulator proteins for the larger proportion of tissue-matched samples in the tumor databank—with the collection of drugs and compounds. The transcriptome of the perturbed cell lines is profiled at low cost by PLATE-Seq. The process begins with the expression profile of a single patient sample, which is compared against the tumor databank to generate a tumor gene expression signature. This signature is interpreted by VIPER using a context-matched interactome to identify the set of most dysregulated proteins, which constitute the regulators of the tumor cell state—the tumor checkpoint. These proteins are then aligned against the drugs' and compounds' MoA database, to prioritize compounds able to invert the activity pattern of the tumor checkpoint.

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Clinical and Translational Impact and Applications

In this landmark perspective, we introduced the **Oncotecture** hypothesis, which postulates the existence of Master Regulator (MR) proteins responsible for implementing and homeostatically maintaining cell tumor state by canalizing the effect of somatic mutations, detrimental germline variants, and aberrant microenvironment signals.

PERSPECTIVES

OPINION

The recurrent architecture of tumour initiation, progression and drug sensitivity

Andrea Califano and Mariano J. Alvarez

Abstract | Recent studies across multiple tumour types are starting to reveal a recurrent regulatory architecture in which genomic alterations cluster upstream of functional master regulator (MR) proteins, the aberrant activity of which is both necessary and sufficient to maintain tumour cell state. These proteins form small, hyperconnected and autoregulated modules (termed tumour checkpoints) that are increasingly emerging as optimal biomarkers and therapeutic targets. Crucially, as their activity is mostly dysregulated in a post-translational manner, rather than by mutations in their corresponding genes or by differential expression, the identification of MR proteins by conventional methods is challenging. In this Opinion article, we discuss novel methods for the systematic analysis of MR proteins and of the modular regulatory architecture they implement, including their use as a valuable reductionist framework to study the genetic heterogeneity of human disease and to drive key translational applications.

There is an intriguing yet largely unexplored paradox in cancer. On the one hand, transcriptional programmes are highly conserved across samples that represent the same tumour subtype[1] — even compared with normal tissue (FIG. 1a) — suggesting the existence of relatively stable tumour states. On the other hand, the genetic and epigenetic alterations (henceforth referred to as genomic alterations) that determine these states are remarkably heterogeneous on a sample by sample basis (BOX 1).

Consistent with these observations, although genomic alterations represent valuable predictors of targeted inhibitor sensitivity against the corresponding oncoproteins, they have proved less effective in stratifying more general properties of cancer, such as tumour subtype, metastatic potential and clinical outcome[2,3], which are more often captured by transcriptional or proteomic profiles[4,5] (BOX 1). For example, immunohistochemistry assays, as well as gene expression-based tests such as Oncotype DX and MammaPrint[6], are routinely used in the clinic to assess the risk of breast cancer recurrence following hormonal therapy.

The ability of tumours to present similar transcriptional signatures, despite having radically distinct somatic mutational profiles, suggests the existence of regulatory mechanisms that are responsible for decoupling (that is, buffering) the tumour cell state from its genetic, epigenetic and signalling determinants[7,8]. Specifically, this Opinion article provides an overview of the emergence of a universal gene-regulatory architecture (henceforth referred to as oncotecture), in which a handful of master regulator (MR) proteins — organized into tightly autoregulated tumour checkpoint modules — implement and maintain the transcriptional state of a tumour cell largely independent of initiating events, as well as of endogenous and exogenous perturbations[7,8]. Much like a highway checkpoint, these modular structures canalize the effect of mutations and other aberrant signals in their upstream pathways to implement key downstream transcriptional programmes (FIG. 1b). Thus, although individual MR proteins are highly tumour specific[9–11], the resulting tumour checkpoint oncotecture is virtually tumour independent[7].

The term MR was initially introduced by developmental biologists to describe gene products (mostly transcription factors) at the top of regulatory hierarchies that are both necessary and sufficient to induce morphogenesis[12] or specific steps in lineage differentiation[13]. By contrast, cancer biologists have adopted a looser definition of the term to indicate genetic determinants or functional drivers, the aberrant activity of which is sufficient — yet not strictly necessary — to induce transformation. According to the latter, *TP53* (which encodes p53) and *ERBB2* (which encodes HER2) are often presented as cancer MRs[14,15], even though many alternative genomic alterations may induce transcriptional tumour states that are virtually indistinguishable from those in which those genes are mutated (BOX 1). For instance, several tumours classified as basal-like or HER2+ by gene expression profile analysis lack the hallmark genetic alterations in *TP53* and *ERBB2*, respectively[16]. In this Opinion article, not only do we adopt the stricter MR definition from developmental biology but we further require MRs to directly regulate the transcriptional state of the tumour cell (FIG. 1b). Under this definition, a classic cancer driver such as p53 may not be considered a MR unless it is necessary and sufficient to implement a subset of tumours with a common transcriptional signature and it directly participates in its signature regulation. By contrast, the MYC proto-oncoprotein, the locus of which is translocated in 100% of Burkitt lymphomas[17] and which regulates their signature[18], would emerge as a bona fide MR protein in that context.

This stricter terminology is not arbitrary. Instead, it is necessary to address the currently ambiguous use of the term in the literature, thus making identification of MR proteins a more rational and systematic process. Consistently, it separates the methodologies and studies discussed in this Opinion article from those aimed at identifying more loosely defined functional drivers or genetic determinants of cancer, for instance, by integrating mutational and gene expression

There are several crucial unaddressed issues implied by such a tumour-independent oncotecture. These include whether tumour heterogeneity supports the plastic coexistence of distinct cancer cell states in the same tumour, which may require pharmacological targeting of multiple distinct tumour checkpoint MRs, for example, using alternating treatment schedules. For instance, in glioma, mesenchymal and proneural subtype cells coexist plastically in the same tumour[19], even though these states have been shown to be regulated by orthogonal tumour checkpoints[10]. In addition, it is unclear how many distinct tumour checkpoints may coexist even in the same tumour cell. For instance, tumour checkpoints associated with proliferation and epithelial-to-mesenchymal transition are probably distinct, given the serial nature of the cancer initiation and progression steps associated with their activation. It is thus possible that a relatively small number of molecularly distinct tumour checkpoints exist and that their activation in distinct tumour subtypes depends on distinct genetic alteration patterns. Combinatorial superposition of two or more of these mechanisms in each individual tumour may thus explain the large variety of reported tumour subtypes and of associated clinical phenotypes and drug sensitivities.

Ultimately, we propose that tumour checkpoint and MRs may be leveraged to simultaneously identify crucial tumour dependencies, as well as the specific drugs and drug combinations that are optimally suited to inducing their collapse, on the basis of an individual sample and even an individual cell. This would have obvious application to complementing oncogene addiction and immune checkpoint paradigms in precision medicine.

Andrea Califano is at the Department of Systems Biology, Columbia University, and the Departments of Biomedical Informatics, Biochemistry and Molecular Biophysics, JP Sulzberger Columbia Genome Center, Herbert Irving Comprehensive Cancer Center, Columbia University, New York, New York 10032, USA.

Mariano J. Alvarez is at DarwinHealth, Inc., 3960 Broadway, Suite 540, New York, New York 10032, USA.

Correspondence to A.C. andrea.califano@columbia.edu

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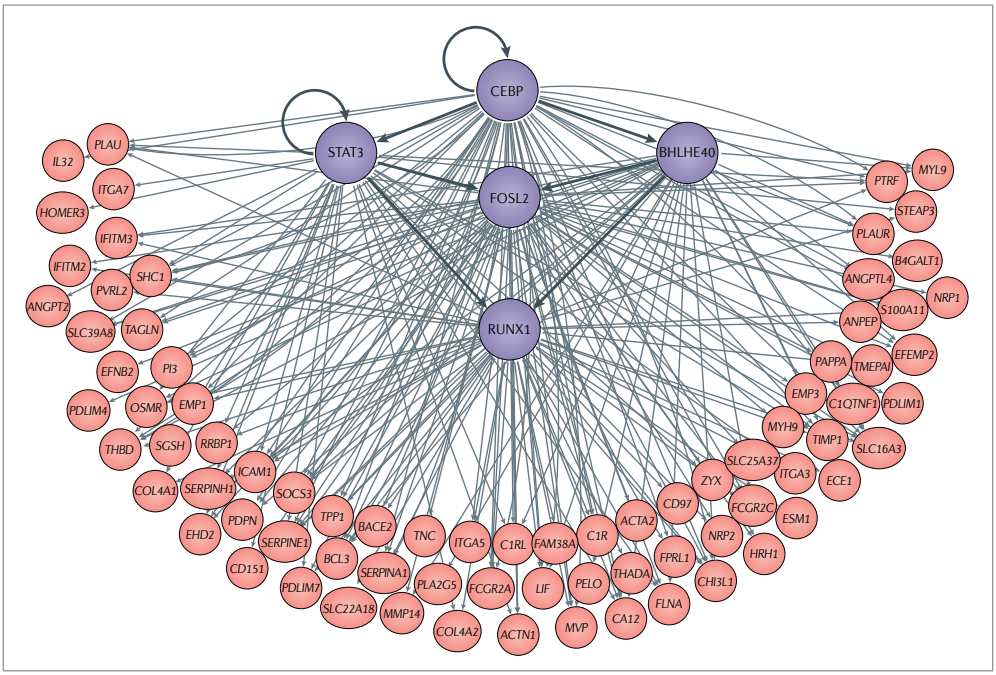

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Clinical and Translational Impact and Applications

This manuscript reports on a clinical trial in metastatic breast cancer in which patients were treated with ricolinostat (HDAC6i) + nab-paclitaxel, on the basis of HDAC6 being identified as a critical **OncoTarget**-identified dependency of these tumors in a previous publication (Putcha et al. 2015 Breast Cancer Res 17, 149). The trial showed 100% consistency with the **OncoTarget** prediction as all patients with high **VIPER**-assessed HDAC6 activity showed clinical response, while all those with low activity rapidly progressed (AUC = 1). This was (a) not detectable at the gene expression level and (b) opposite to the outcome trend reported for untreated HDAC6+ and HDAC6- tumors

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Network-based assessment of HDAC6 activity predicts preclinical and clinical responses to the HDAC6 inhibitor ricolinostat in breast cancer

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Tizita Z. Zeleke[1,14], Qingfei Pan[2,14], Codruta Chiuzan[3], Maika Onishi[4], Yuxin Li[5,6], Haiyan Tan[6], Mariano J. Alvarez[7,8], Erin Honan[9], Min Yang[10], Pei Ling Chia[1], Partha Mukhopadhyay[1], Sean Kelly[9], Ruby Wu[9], Kathleen Fenn[9], Meghna S. Trivedi[9], Melissa Accordino[9], Katherine D. Crew[9], Dawn L. Hershman[9], Matthew Maurer[11], Simon Jones[12], Anthony High[6], Junmin Peng[5,6], Andrea Califano[7], Kevin Kalinsky[9,14] ✉, Jiyang Yu[2,14] ✉ & Jose Silva[13,14] ✉

Inhibiting individual histone deacetylase (HDAC) is emerging as well-tolerated anticancer strategy compared with pan-HDAC inhibitors. Through preclinical studies, we demonstrated that the sensitivity to the leading HDAC6 inhibitor (HDAC6i) ricolinstat can be predicted by a computational network-based algorithm (HDAC6 score). Analysis of ~3,000 human breast cancers (BCs) showed that ~30% of them could benefice from HDAC6i therapy. Thus, we designed a phase 1b dose-escalation clinical trial to evaluate the activity of ricolinostat plus nab-paclitaxel in patients with metastatic BC (MBC) (NCT02632071). Study results showed that the two agents can be safely combined, that clinical activity is identified in patients with $HR^+/HER2^-$ disease and that the HDAC6 score has potential as predictive biomarker. Analysis of other tumor types also identified multiple cohorts with predicted sensitivity to HDAC6i's. Mechanistically, we have linked the anticancer activity of HDAC6i's to their ability to induce c-Myc hyperacetylation (ac-K148) promoting its proteasome-mediated degradation in sensitive cancer cells.

A total of five predefined doses of ricolinostat were selected for the dose-escalation process (Fig. 3a). Seventeen patients were accrued between March 2016 and February 2018. Of these, 16 patients had an evaluable disease, as one patient no longer wishing to participate in the trial dropped out at cycle 2 in the absence of any related toxicity. In the 16 evaluable patients, the median age was 57.5 years (range: 41–78); 14 were female (87.5%), 3 had triple-negative MBC and 13 had $HR^+/HER2^-$ MBC. The median number of prior lines was 3 (range: 0–10) (Extended Data Fig. 3a). The first patient started at 120 mg/m^2 daily, the second patient started at 180 mg/m^2 daily and the remaining 14 patients were treated at 240 mg/m^2 daily. No DLTs were seen in the DLT window of 8 weeks (first two cycles) and thus the MTD was not reached. Grade III events related to nab-paclitaxel included neutropenia ($n = 1$), peripheral neuropathy ($n = 1$) and 1 grade IV neutropenia. Grade III syncope related to ricolinostat was observed in 2 patients (Supplementary Tables 4–6). All of these events occurred after the DLT window. In the 16 evaluable patients, the following were best responses: 2 partial response (PR), 10 stable disease (SD), and 4 progressive disease (PD; 2 TNBC, 2 $HR^+/HER2^-$) (Fig. 3b). All patients had measurable disease (Fig. 3b), except for one evaluable patient without target lesions who was reported to have SD for 9 months. Three patients who previously received a taxane for metastatic disease achieved SD with ricolinostat plus nab-paclitaxel. One patient with SD remains on treatment since Feb 2018 (17 months). The CBR was 31.25%: 5/16 patients (2 PR and 3 SD >6 months). All of these patients were diagnosed with $HR^+/HER2^-$ MBC, except for one patient with TNBC and SD. Median PFS was 5.3 months (95% confidence interval (CI): 4.45–11.0) (Supplementary Tables 7–9).

We were able to obtain tumor specimens in the form of formalin-fixed paraffin-embedded (FFPE) sections with >50% in tumor content for 10 of the 16 evaluable patients (3 achieving PD and 7 showing SD or PR). RNA was obtained from these samples, subject to genome-wide RNA-seq, and the expression profiles obtained were used to calculate the HDAC6 scores. Interestingly, when we compared the HDAC6 scores between patients showing PD (nonresponders) and those with either SD or PR (responders), a statistically significant higher HDAC6 score was seen in responder patients ($P = 2.1 \times 10^{-5}$; Extended Data Fig. 4a). HDAC6 score analysis integrating our clinical study with TCGA, METABRIC and IBC cohorts confirmed our previous hypothesis that patients with $HR^+/HER2^-$ BC respond better than those with TNBC (Fig. 3c). Further, we used receiver operating characteristic (ROC) curves[29] to characterize the sensitivity/specificity of the HDAC6 score using the trial data. Briefly, ROC curve analysis is a graphical plot that illustrates the diagnostic ability of a binary classifier system as its discrimination threshold is varied. The best cutoff value can be calculated by ROC analysis for continuous variables to predict dichotomous variables with the best sensitivity and specificity. In this analysis, the ROC curve analysis for the HDAC6 score achieved an area under the curve (AUC) of 1.0 (Fig. 3d). Although the perfect AUC was likely influenced by the small number of patients, at a cutoff value of −0.36, the HDAC6 score gave rise to a clear separation between responders and nonresponders with 100% accuracy, and it outperformed the subtype ($HR^+/HER2^-$ or TNBC) that had an accuracy of 80% (2 out of 10 patients were mispredicted, including 1 with $HR^+/HER2^-$ and 1 with TNBC). Finally, we examined the predictive power of the HDAC6 score for patient prognosis, measured by PFS. We classified the patients into low and high HDAC6 score groups using the cutoff of −0.36 calculated by ROC analysis. Patients with a high HDAC6 score had a median PFS of 6.51 months (95% CI: 5.19–NA (NA= not enough events to calculate)), which was significantly better ($P = 8.0 \times 10^{-4}$; Fig. 3e) than patients with low HDAC6 score who had a median PFS of 1.84 months (95% CI, 1.08–NA).

Transitioning any molecular biomarker to the clinic requires a Clinical Laboratory Improvement Amendments (CLIA)-certified test. The Darwin OncoTarget test, which is based on the VIPER (Virtual Inference of Protein-activity by Enriched Regulon analysis) algorithm[30], was developed precisely to compute the activity of druggable proteins in cancer patients, based on the expression of their ARACNe-inferred transcriptional targets. The test has recently received CLIA certification from the New York State Department of Health[31]. As a result, we assessed whether HDAC6 activity measured via this clinical-grade test was also predictive of patient sensitivity to the ricolinostat/nab-paclitaxel combination therapy in the phase 1b study. Consistent with the results discussed in the previous sections, the HDAC6 score measured by Darwin OncoTarget was equally effective in stratifying patient sensitivity ($P = 9.4 \times 10^{-5}$; Extended Data Fig. 4b), achieving a classification of the seven responders and three nonresponders with an AUC of 0.9 (95% CI: 0.68–1.0) based on ROC analysis (Extended Data Fig. 4c).

Because of the nature of the phase 1b trials, our study did not include a paclitaxel-only control group. Thus, to investigate if the correlation between the HDAC6 score and the response to ricolinostat plus nab-paclitaxel in the trial was influenced by taxanes, we investigated a publicly available series of BCs ($n = 106$) treated only with paclitaxel[32]. As expected, the distribution of the HDAC6 scores in the series mimicked the results described for TCGA and METABRIC, and the $HR^+/HER2^-$ and luminal B subtypes had the highest values, whereas the triple-negative and basal subtypes showed the lowest ones (Extended Data Fig. 5a,b). Importantly the HDAC6 scores showed no correlation with pathologic response to paclitaxel (Extended Data Fig. 5c) or with the patient survival (Extended Data Fig. 5d). Thus, these results demonstrate that the correlation between the HDAC6 score and the response to treatment is linked to the use of ricolinostat.

High HDAC6 scores are found in a variety of human cancers

Because our studies confirmed the prognostic value of the HDAC6 score in human patients, we decided to systematically assess the HDAC6 scores across a large repertoire of human primary malignancies and cancer cell lines. Specifically, we analyzed >10,000 gene expression profiles, representing 32 molecularly distinct human malignancies represented in the TCGA database (https://www.cancer.gov/tcga). First, we generated tumor-specific HDAC6 regulons using the same approach successfully tested in BC and used them to calculate the HDAC6 scores for all TCGA samples in a cancer-type-specific manner. As expected, the number of genes that overlapped among the different tumor types was highly significant, although tumor-type-specific differences were also noticeable (Extended Data Fig. 6a and Supplementary Tables 10 and 11). Next, we aimed to investigate whether a correlation exists between the HDAC6 scores and the response to therapy in other tumor types. For this, we performed dose–response studies to assess ricolinostat IC50 in 58 additional cancer lines, representing 11 different tumor types. Notably, confirming the BC-specific findings, a significant anticorrelation was detected between HDAC6 score and IC_{50} ($R = -0.44$, $P = 5.2 \times 10^{-4}$) (Fig. 4a, Extended Data Fig. 6b,c and Supplementary Table 12). Finally, we assessed the HDAC6 scores in 1,156 different cancer cell lines available in the CCLE cohort, representing 20 tumor types[17], as well as in 32 primary tumors (TCGA database) (Fig. 4b,c and Supplementary Table 13). We also compared the HDAC6 score in cancer cell lines and primary tumors. We used the 20 cancer types with data for both cohorts and reranked each cancer type by HDAC6 score in each cohort separately. Then, we visualized the ranks of the 20 cancer types in a scatter plot and used the Spearman method to calculate the correlation (Fig. 4d). Notably, we observed a strong correlation of the ranks between cell lines and primary samples, showing the consistency of HDAC6 score distribution in these two cohorts and supporting the use of cell lines to investigate the response to an HDAC6i.

In addition to their direct effect on cancer cells, HDAC6i's may have additional effects on the tumor microenvironment. Thus, we evaluated the expression profiles of the TCGA-BRCA samples using the algorithm ESTIMATE[33] to predict infiltration of immune cells. Here, we found a negative correlation between immune infiltration and HDAC6 score (Extended Data Fig. 7a). Although this correlation is not surprising due to the association of higher HDAC6 scores and lower immune scores

HDAC6 score inference by VIPER

HDAC6 relative protein activity based on our next-generation HDAC6 BC regulon was inferred by the VIPER algorithm implemented in the Darwin OncoTarget test, which has been approved by the NYS Department of Health CLIA/CLEP Validation Unit as an offering in the category of 'Molecular and Cellular Tumor Markers for Oncology' (Neal, Michael, Assay Validation Review, Wadsworth Center, NY State Department of Health, PFI: 7313, Project ID: 63859, March 8, 2019).

Overlaps of cancer-type-specific HDAC6 regulons

The HDAC6 regulons of 32 TCGA cancer types inferred by SJARACNe, as described above, were summarized in Supplementary Table 10. The regulon overlapping statistics, including the size of overlapped regulon genes and the P value of Fisher's exact test, was summarized in Supplementary Table 11. The scatter plot was made by R package ggplot2 (v3.3.0).

PFS analysis against HDAC6 score in the clinical trial

The PFS in month was calculated from the dates of the trial assignment and disease progression or last follow-up by the 'difftime' function in lubridate (v1.7.8). The PFS analysis of the clinical trial against HDAC6 score, including the Cox model fitting and Kaplan–Meier plot, was performed by using the R package survminer (v0.4.6). The HDAC6 score 'high' and 'low' patients were defined at the cutoff of the mean of HDAC6 scores.

ROC curve analysis of HDAC6 score in the clinical trial

The ROC curve analysis to evaluate the performance of HDAC6 score in predicting clinical response of patients, including statistics and plot, was performed with the R package pROC (v1.6.2)[70]. We used −0.36 as

Code availability

The codes for the HDAC6 score calculation and other analyses are freely available at https://github.com/jyyulab/HDAC6-score

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Independent "News and Views" Analysis of DarwinOncoTarget HDAC6 Scoring Effectiveness in Metastatic Breast Cancer Characterized as "a Roadmap for How to Advance Cancer Therapy" in *Nature Cancer* by International Group of Cancer Researchers

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Network-based assessment of HDAC6 activity predicts preclinical and clinical responses to the HDAC6 inhibitor ricolinostat in breast cancer

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Tizita Z. Zeleke[1,14], Qingfei Pan[2,14], Codruta Chiuzan[3], Maika Onishi[4], Yuxin Li[5,6], Haiyan Tan[6], Mariano J. Alvarez[7,8], Erin Honan[9], Min Yang[10], Pei Ling Chia[1], Partha Mukhopadhyay[1], Sean Kelly[9], Ruby Wu[9], Kathleen Fenn[9], Meghna S. Trivedi[9], Melissa Accordino[9], Katherine D. Crew[9], Dawn L. Hershman[9], Matthew Maurer[11], Simon Jones[12], Anthony High[6], Junmin Peng[5,6], Andrea Califano[7], Kevin Kalinsky[8,14] ✉, Jiyang Yu[2,14] ✉ & Jose Silva[13,14] ✉

the correct patient population. Critically, the HDAC6 score developed in this manuscript provides an effective predictive biomarker to identify patients most likely to benefit from HDAC6i therapy. In terms of immediate clinical translation, we have shown that the New York CLIA-certified Darwin OncoTarget test produces HDAC6 scores that are highly predictive of patient sensitivity to ricolinostat plus nab-paclitaxel combination therapy.

Transitioning any molecular biomarker to the clinic requires a Clinical Laboratory Improvement Amendments (CLIA)-certified test. The Darwin OncoTarget test, which is based on the VIPER (Virtual Inference of Protein-activity by Enriched Regulon analysis) algorithm[30], was developed precisely to compute the activity of druggable proteins in

INDEPENDENT COMMENTARY on IMPLICATIONS of HDAC6 REPORT

News & views

Cancer biomarkers

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HDAC6 score: to treat or not to treat?

Joschka Hey, Maria Llamazares Prada & Christoph Plass

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Developing approaches to identify patients who may benefit from specific treatments is an important area of research. A study now defines an HDAC6 score to predict the response of patients with breast cancer to the HDAC6 inhibitor ricolinostat and characterizes its anti-tumor effects with preclinical mechanistic work and a phase 1b clinical trial.

samples and 47 breast cancer cell lines. This analysis revealed high HDAC6 activity for approximately 30% of all primary breast cancers, indicating that there may be preferential sensitivity to HDAC6 inhibition. Breast cancers with high HDAC6 scores were enriched in the clinical subtypes HR^+ and $HER2^+$, as well as in molecular luminal-B and HER2. Further characterization of a panel of breast cancer cell lines revealed an inverse correlation between the half-maximal inhibitory concentration (IC_{50}) of ricolinostat and HDAC6 scores along with a reduction of cell survival in ricolinostat-sensitive cells, characterized by a high HDAC6 score.

To evaluate the anticancer activity of ricolinostat in vivo, Zeleke et al.[9] further assessed the efficacy of ricolinostat in combination with

Altogether, Zeleke et al.[9] provide a roadmap for how to advance cancer therapy. In this study, they generated substantial evidence advocating for ricolinostat treatment of patients with breast cancer with a high HDAC6 score and provided the foundation for further studies on other tumor types with a high HDAC6 score. This work is the result of a detailed mechanistic understanding of the function of HDAC6, the computational network analysis evaluating HDAC6 activity and preclinical and clinical work. The tools and knowledge to

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Clinical and Translational Impact and Applications

VIPER and **DarwinOncoTreat** methodologies are used to identify immune subpopulations at the single cell level from 36 patients across four tumor types. By comparing peripheral vs. tumor-resident regulatory T cells (Tregs)–a highly immunosuppressive subpopulations–on a patient-by-patient basis, the analysis identified 17 MRs of Treg tumor residency, 8 of which were experimentally validated in chimeric mouse assays. CRISPR-mediated silencing of the top MR, TRPS1, induced spontaneous tumor remission in highly aggressive syngeneic tumor models. Moreover, TRPS1 silencing synergized with PD1 inhibitors.

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Systematic elucidation and pharmacological targeting of tumor-infiltrating regulatory T cell master regulators

Aleksandar Obradovic,[1,2,16] Casey Ager,[1,3,16] Mikko Turunen,[2,16] Thomas Nirschl,[4] Mohsen Khosravi-Maharlooei,[1] Alina Iuga,[14] Christopher M. Jackson,[5] Srinivasan Yegnasubramanian,[4] Lorenzo Tomassoni,[2] Ester Calvo Fernandez,[2] Patrick McCann,[9] Meri Rogava,[9] Angelo M. DeMarzo,[4,6,13] Christina M. Kochel,[4] Mohamad Allaf,[6] Trinity Bivalacqua,[6] Michael Lim,[7] Ronald Realubit,[2,8,9] Charles Karan,[2,8,9] Charles G. Drake,[1,4,15] and Andrea Califano[2,8,9,10,11,12,17,*]

[1]Columbia Center for Translational Immunology, Irving Medical Center, New York, NY, USA
[2]Department of Systems Biology, Columbia University Irving Medical Center, New York, NY, USA
[3]Department of Hematology Oncology, Columbia University Irving Medical Center, New York, NY, USA
[4]Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[5]Department of Neurosurgery, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[6]Department of Urology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[7]Department of Neurosurgery, Stanford School of Medicine, Palo Alto, CA, USA
[8]J.P. Sulzberger Columbia Genome Center, Columbia University, New York, NY, USA
[9]Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center, New York, NY, USA
[10]Department of Medicine, Columbia University Irving Medical Center, New York, NY, USA
[11]Department of Biochemistry and Molecular Biophysics, Columbia University Irving Medical Center, New York, NY, USA
[12]Department of Biomedical Informatics, Columbia University Irving Medical Center, New York, NY, USA
[13]Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[14]Department of Pathology, UNC School of Medicine, Chapel Hill, NC, USA
[15]Present address: Janssen Research and Development, Springhouse, PA, USA
[16]These authors contributed equally
[17]Lead contact
*Correspondence: ac2248@cumc.columbia.edu
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SUMMARY

Due to their immunosuppressive role, tumor-infiltrating regulatory T cells (TI-Tregs) represent attractive immuno-oncology targets. Analysis of TI vs. peripheral Tregs (P-Tregs) from 36 patients, across four malignancies, identified 17 candidate master regulators (MRs) as mechanistic determinants of TI-Treg transcriptional state. Pooled CRISPR-Cas9 screening *in vivo*, using a chimeric hematopoietic stem cell transplant model, confirmed the essentiality of eight MRs in TI-Treg recruitment and/or retention without affecting other T cell subtypes, and targeting one of the most significant MRs (Trps1) by CRISPR KO significantly reduced ectopic tumor growth. Analysis of drugs capable of inverting TI-Treg MR activity identified low-dose gemcitabine as the top prediction. Indeed, gemcitabine treatment inhibited tumor growth in immunocompetent but not immunocompromised allografts, increased anti-PD-1 efficacy, and depleted MR-expressing TI-Tregs *in vivo*. This study provides key insight into Treg signaling, specifically in the context of cancer, and a generalizable strategy to systematically elucidate and target MR proteins in immunosuppressive subpopulations.

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STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Cell lines
  - Primary cell cultures
  - Animals
- METHOD DETAILS
  - Clinical sample collection, sorting, and RNA-sequencing
  - Gene expression and VIPER analysis
  - Random Forest Feature Selection
  - CRISPR KO library design
  - CRISPR KO oligo synthesis and library cloning
  - Lentiviral packaging of the sgRNA library
  - sgRNA library transductions into hematopoietic LSK cells
  - CRISPR validation in chimeric immune editing model
  - Genomic DNA extraction and preparation of NGS libraries
  - *In vivo* CRISPR KO-screen analysis
  - CRISPR KO-based tumor growth experiments
  - ORF cloning for transcription factor overexpression/reprogramming assay
  - Transcription factor overexpression / reprogramming assay (scRNA-Seq)
  - High-throughput Treg-directed drug screening
  - Drug-based tumor growth experiments
  - *In vitro* Treg suppression assay
  - Histology
  - High dimensional spectral flow cytometry immune profiling
  - Single-cell RNA-seq profiling of gemcitabine effect on TI-Tregs
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2023.04.003.

of the TI-TREG MRs. This study was also supported by the NIH/NCI SPORE in Prostate Cancer (P50CA58236) and the US Department of Defense Prostate Cancer Research Program (PCRP) Prostate Cancer Biospecimen Network Site (W81XWH-18-2-0015).

AUTHOR CONTRIBUTIONS

A.O. conceived of, coordinated, and assisted in performing experiments; performed data analysis; and co-wrote the manuscript. C.A. conceived of, designed, and performed all experiments; designed all flow cytometry panels; and co-wrote the manuscript. M.T. conceived of and aided in design of experiments and optimized and performed all CRISPR-based protocols. M.K.-M. assisted in hematopoietic stem cell transplantation. C.M.J., T.N., C.M.K., M.A., T.B., A.M.D., and M.L. recruited patients and coordinated collection of tumor and peripheral blood specimens for sorted T cell bulk RNA-seq. C.K. ran the high-throughput drug screen PLATE-seq assay. A.C. and C.G.D. conceived of the project, advised on overall experimental design and data analysis procedure, and co-wrote the manuscript.

DECLARATION OF INTERESTS

C.G.D. is a co-inventor on patents licensed from JHU to BMS and Janssen; has served as a paid consultant to AZ Medimmune, BMS, Pfizer, Roche, Sanofi Aventis, Genentech, Merck, and Janssen; and has received sponsored research funding to his institution from BMS IioN and Janssen. A.C. is founder, equity holder, consultant, and director of DarwinHealth, Inc., which has licensed IP related to these algorithms from Columbia University. Columbia University is an equity holder in DarwinHealth, Inc. S.Y. has received sponsored research support to his institution from Celgene/BMS, Janssen, and Cepheid/Danaher and has served as a paid consultant to Cepheid/Danaher. A.O., C.A., C.G.D., and A.C. are co-inventors on US provisional patent no. 63/188,970, "Therapeutic modulation of regulatory T cells through master regulatory protein targeting," which relates to the work described here.

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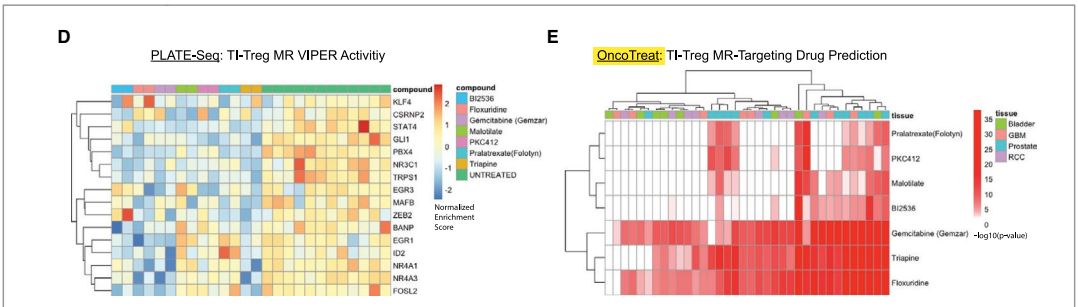

Figure 3. High-throughput drug screening platform identifies potential drug candidates with tumor-Treg-directed toxicity

(A) Experimental design of high-throughput Treg-directed drug toxicity screen.

(B) Results from initial set of 1,554 FDA-approved and investigational oncology compounds screened at a single dose for peripheral Treg growth inhibition, with 195 compounds showing >60% inhibition at 5 μM.

(C) Viability results of the PLATE-seq screen, where human tumor Tregs were assessed for growth inhibition on sorted tumor Tregs at peripheral-Treg EC_{20} dose, resulting in seven drugs with higher toxicity in TI-Tregs relative to P-Tregs. Data are shown as percentage viability for each drug vs. DMSO control.

(D) Heatmap of VIPER protein activity for tumor vs. peripheral Treg MRs defined in Figures 1E and 1F comparing transcriptional effect of drugs in (C) vs. untreated control, with downregulation of nearly all identified MRs by these drugs.

(E) Patient-by-patient drug predictions according to inversion of patient tumor Treg vs. peripheral Treg protein activity signature by drug-treatment protein activity signature. Each drug predicted to invert tumor Treg signature with −log10(Bonferroni-corrected p value) <0.01 in a particular patient is colored red. Patients are grouped by tumor type. The plot is subset to show only drugs identified by tumor Treg growth screen in (C), with columns colored by tumor type and clustered by unsupervised hierarchical clustering.

See also Figures S3 and S4.

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CANCER DISCOVERY

DARWIN HEALTH™
PRECISION THERAPEUTICS for CANCER MEDICINE

Clinical and Translational Impact and Applications

This real time, drug prioritization study, conducted in the setting of castration-resistant, metastatic, and neuroendocrine prostate cancer uses **DarwinHealth's VIPER**-based **DarwinOncoTreat** analytical platform, in a rapid-turnaround context, for identifying and predicting drugs that will enhance the efficacy of clinically relevant drugs, in particular, the PD-1 inhibitor nivolumab and the AR inhibitor enzalutamide in individual patients, based on Master Regulator (MR) signatures and leveraging drug perturbation profiles.

RESEARCH ARTICLE

OncoLoop: A Network-Based Precision Cancer Medicine Framework

Alessandro Vasciaveo[1], Juan Martín Arriaga[2], Francisca Nunes de Almeida[2], Min Zou[2], Eugene F. Douglass Jr[1], Florencia Picech[2], Maho Shibata[3,4,5], Antonio Rodriguez-Calero[6,7], Simone de Brot[8], Antonina Mitrofanova[1], Chee Wai Chua[3,4,5], Charles Karan[1,9], Ronald Realubit[1,9], Sergey Pampou[1,9], Jaime Y. Kim[2], Stephanie N. Afari[2], Timur Mukhammadov[2], Luca Zanella[1], Eva Corey[10], Mariano J. Alvarez[1,11], Mark A. Rubin[6,12], Michael M. Shen[1,3,4,5,13], Andrea Califano[1,3,9,13,14,15], and Cory Abate-Shen[1,2,3,5,13,16]

ABSTRACT Prioritizing treatments for individual patients with cancer remains challenging, and performing coclinical studies using patient-derived models in real time is often unfeasible. To circumvent these challenges, we introduce OncoLoop, a precision medicine framework that predicts drug sensitivity in human tumors and their preexisting high-fidelity (cognate) model(s) by leveraging drug perturbation profiles. As a proof of concept, we applied OncoLoop to prostate cancer using genetically engineered mouse models (GEMM) that recapitulate a broad spectrum of disease states, including castration-resistant, metastatic, and neuroendocrine prostate cancer. Interrogation of human prostate cancer cohorts by Master Regulator (MR) conservation analysis revealed that most patients with advanced prostate cancer were represented by at least one cognate GEMM-derived tumor (GEMM-DT). Drugs predicted to invert MR activity in patients and their cognate GEMM-DTs were successfully validated in allograft, syngeneic, and patient-derived xenograft (PDX) models of tumors and metastasis. Furthermore, OncoLoop-predicted drugs enhanced the efficacy of clinically relevant drugs, namely, the PD-1 inhibitor nivolumab and the AR inhibitor enzalutamide.

SIGNIFICANCE: OncoLoop is a transcriptomic-based experimental and computational framework that can support rapid-turnaround coclinical studies to identify and validate drugs for individual patients, which can then be readily adapted to clinical practice. This framework should be applicable in many cancer contexts for which appropriate models and drug perturbation data are available.

INTRODUCTION

Systematic prediction of drug efficacy *in vivo* remains a major clinical challenge for most cancer types due, in part, to tumor heterogeneity, which makes it difficult to optimize treatments on an individual basis. This is further compounded by difficulties in establishing patient-derived models that recapitulate the biology and complexity of an individual patient's tumor for coclinical validation. Indeed, for some tumor types, the establishment of patient-derived xenograft (PDX) models can take more than 1 year (1, 2), thus compromising their usefulness for evaluating drug efficacy within a timeframe compatible with patient care, especially in the metastatic setting. Patient-derived organoid (PDO) models have become increasingly more accessible and representative; however, these may not effectively model the tumor microenvironment (3, 4). Although human tumor cell lines are widely available for many cancer types, they rarely represent the full spectrum of tumor phenotypes observed in patients and often have idiosyncratic dependencies, as a result of alterations they accrue to survive *in vitro*. In principle, genetically engineered mouse models (GEMM), which are now widely available for many cancer types (5), may be valuable for studying

[1]Department of Systems Biology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, New York. [2]Department of Molecular Pharmacology and Therapeutics, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, New York. [3]Department of Medicine, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, New York. [4]Department of Genetics and Development, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, New York. [5]Department of Urology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, New York. [6]Department for Biomedical Research, University of Bern, Bern, Switzerland. [7]Institute of Pathology, University of Bern and Inselspital, Bern, Switzerland. [8]COMPATH, Institute of Animal Pathology, University of Bern, Bern, Switzerland. [9]J.P. Sulzberger Columbia Genome Center, Columbia University Irving Medical Center, New York, New York. [10]Department of Urology, University of Washington, Seattle, Washington. [11]DarwinHealth Inc., New York, New York. [12]Bern Center for Precision Medicine (BCPM), Bern, Switzerland. [13]Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center, New York, New York. [14]Department of Biochemistry and Molecular Biophysics, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, New York. [15]Department of Biomedical Informatics, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, New York. [16]Department of Pathology and Cell Biology, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, New York.

Note: A. Vasciaveo, J.M. Arriaga, and F. Nunes de Almeida contributed equally to this work.

Current address for J.M. Arriaga: Department of Oncological Sciences and Department of Urology, Icahn School of Medicine at Mount Sinai, New York, New York; current address for M. Zou, Arvinas, New Haven, Connecticut; current address for E.F. Douglass Jr, Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, Georgia; current address for M. Shibata, Department of Anatomy and Cell Biology and GW Cancer Center, The George Washington University School of Medicine and Health Sciences, Washington, DC; current address for A. Mitrofanova, Department of Health Informatics, Rutgers School of Health Professions, Rutgers Biomedical and Health Sciences, Newark, New Jersey; current address for C.W. Chua, State Key Laboratory of Oncogenes and Related Genes, Renji-Med X Clinical Stem Cell Research Center, Department of Urology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Pudong New District, Shanghai, China; and current address for R. Realubit, Regeneron Pharmaceuticals, Inc., Tarrytown, New York.

Corresponding Author: Andrea Califano, Department of Systems Biology, 1130 Saint Nicholas Avenue, Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY 10032. Phone: 212-851-5183; E-mail: ac2248@cumc.columbia.edu

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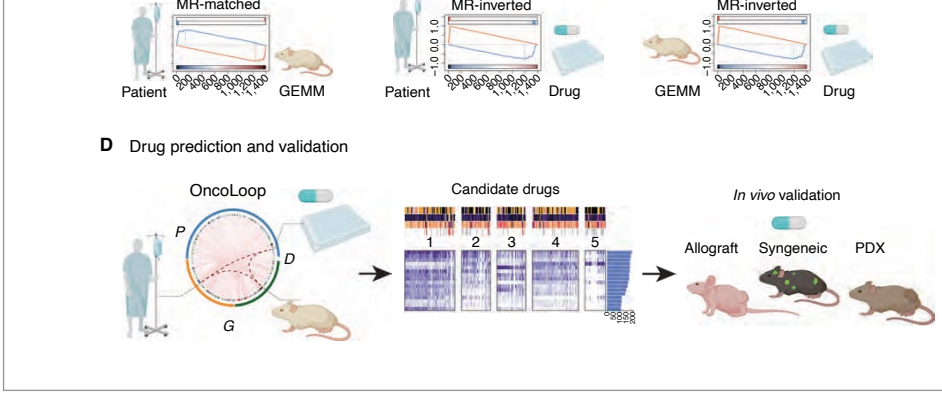

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CURRENT PROTOCOLS

Clinical and Translational Impact and Applications

This invited paper reports on the step-by-step methodologies and technologies that underpin **DarwinOncoDiscovery** cancer drug discovery platform. The protocol systematically identifies, from specific patient tumor samples, the MRs that comprise the tumor checkpoint, then identifies selection process for in vitro and in vivo models that, by recapitulating the patient's tumor checkpoint, constitute the appropriate cell lines and xenografts to further elucidate the tissue context–specific drug mechanism of action (MOA). This proprietary **DarwinHealth** platform permits precise, biomarker-based preclinical validations of drug efficacy, a systematic pipeline that provides optimized drug-tumor alignments and mechanism-based biomarkers to enrich prospective clinical trials with patients likely to respond.

A Patient-to-Model-to-Patient (PMP) Cancer Drug Discovery Protocol for Identifying and Validating Therapeutic Agents Targeting Tumor Regulatory Architecture

Pasquale Laise,[1,2] Gideon Bosker,[1] Andrea Califano,[2,3,4,5,6,7] and Mariano J. Alvarez[1,2,7]

[1]DarwinHealth, New York, New York
[2]Department of Systems Biology, Columbia University Irving Medical Center, New York, New York
[3]Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center, New York, New York
[4]Department of Medicine, Columbia University Irving Medical Center, New York, New York
[5]Department of Biochemistry & Molecular Biophysics, Columbia University Irving Medical Center, New York, New York
[6]Department of Biomedical Informatics, Columbia University Irving Medical Center, New York, New York
[7]Corresponding authors: *ac2248@cumc.columbia.edu*; *malvarez@darwinhealth.com*

Published in the Pharmacology section

The current Achilles heel of cancer drug discovery is the inability to forge precise and predictive connections among mechanistic drivers of the cancer cell state, therapeutically significant molecular targets, effective drugs, and responsive patient subgroups. Although advances in molecular biology have helped identify molecular markers and stratify patients into molecular subtypes, these associational strategies typically fail to provide a mechanistic rationale to identify cancer vulnerabilities. Recently, integrative systems biology methodologies have been used to reverse engineer cellular networks and identify master regulators (MRs), proteins whose activity is both necessary and sufficient to implement phenotypic states under physiological and pathological conditions, which are organized into highly interconnected regulatory modules called tumor checkpoints. Because of their functional relevance, MRs represent ideal pharmacological targets and biomarkers. Here, we present a six-step patient-to-model-to-patient protocol that employs computational and experimental methodologies to reconstruct and interrogate the regulatory logic of human cancer cells for identifying and therapeutically targeting the tumor checkpoint with novel as well as existing pharmacological agents. This protocol systematically identifies, from specific patient tumor samples, the MRs that comprise the tumor checkpoint. Then, it identifies *in vitro* and *in vivo* models that, by recapitulating the patient's tumor checkpoint, constitute the appropriate cell lines and xenografts to further elucidate the

Rodriguez-Barrueco et al., 2015; Walsh et al., 2017), gastroenteropancreatic neuroendocrine tumors (Alvarez et al., 2018), neuroblastoma (Rajbhandari et al., 2018), pancreas (Laise et al., 2020), and prostate carcinoma (Aytes et al., 2013; Aytes et al., 2014), among others (Paull et al., 2021). Moreover, by mechanistically regulating the aberrant transcriptional state of tumors, MR proteins operate downstream, canalizing the effect of most genetic alterations. As such, they can be viewed as a regulatory bottleneck whose targeting cannot be bypassed by additional alterations in upstream pathways (Califano & Alvarez, 2017) (Fig. 1). In contrast to classic approaches for biomarker discovery, based on empirical/statistical associations between features and outcomes, the analysis of regulatory networks provides a mechanistic rationale for the role of MR proteins in implementing and maintaining the tumor cell state and as such represents a method for identifying ideal candidate therapeutic targets and biomarkers.

THE PATIENT-TO-MODEL-TO-PATIENT PROTOCOL

In this article, we describe the patient-to-model-to-patient (PMP) protocol for tumor checkpoint–based drug discovery developed at DarwinHealth, based on algorithms and computational tools developed in the Califano Lab at Columbia University and licensed exclusively for commercial use and cancer drug discovery to DarwinHealth. The first step of the PMP protocol relies on the systematic analysis of gene expression profiles—RNA sequencing—of tumor samples from cancer **P**atients and the identification of MRs constituting the tumor checkpoint. Based on the conservation of the patient's tumor checkpoint—which consists of a signature characterized by MR protein activity levels—the PMP protocol identifies the most appropriate cell line and patient-derived xenograft (PDX) **M**odels. The selected cell line models are then used for drugs' context-specific mechanism of action (MOA) elucidation and engineered or PDX models. Finally, the PMP protocol returns to cancer **P**atients, leveraging the context-specific drug MOA/tumor checkpoint–based biomarkers to enrich clinical trial cohorts with patients most likely to show clinical response (tumor suppression) to the predicted pharmacologic intervention (see Fig. 2 for a schematic representation).

Part 1 of the PMP Protocol: Systematic Identification of Patients' Master Regulator Proteins and Tumor Checkpoints

A key aspect of the PMP protocol is the identification of non-oncogene-based tumor dependencies directly from *patients*' tumor samples, thereby avoiding the potential biased and idiosyncratic results commonly associated with the use of animal or cell culture–based tumor models (Fig. 2A).

MR elucidation in specific, patient-derived tumors requires gene expression profiling of fresh-frozen (recommended) or formalin-fixed tissue from tumor samples obtained from biopsies or tumor tissue following tumor resection/excision. Typically, such gene expression profiles require a minimum sequencing depth of 5 million reads aligned to the reference transcriptome for samples with a tumor cell content >60% (Alvarez et al., 2018). Satisfying these conditions is required because potential contamination of the expression profile with normal cells embedded in the tumor stroma dilutes the signal, thereby limiting our ability to characterize the transcriptional identity of the tumor cells specifically and therefore its MRs. We have previously shown that a sequencing depth of 5 million reads is adequate for protein activity quantification using the VIPER algorithm (see below). Moreover, it has been shown that VIPER-based results quantifying protein activity levels are extremely robust despite limited sequencing depth, producing virtually identical results once the sequence depth is higher than 2 million aligned reads, with Pearson's correlation (R > 0.98) when comparing

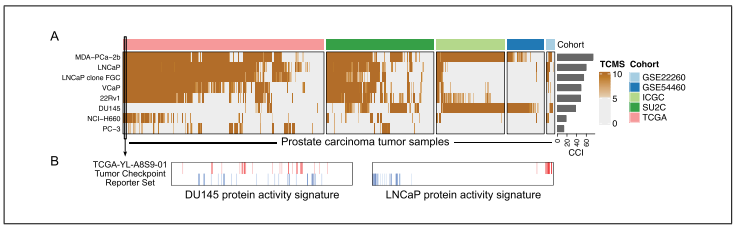
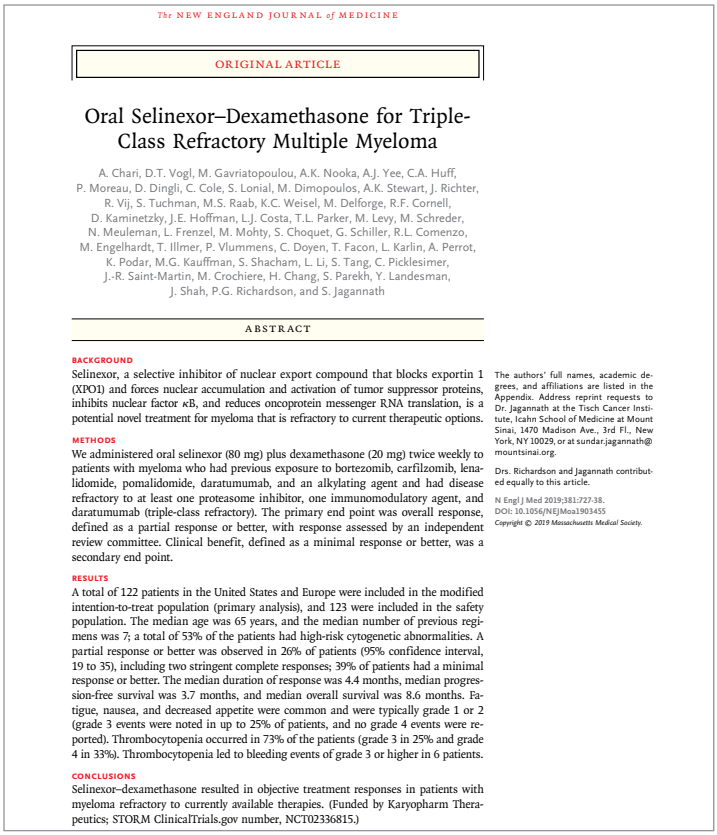


Figure 3 DarwinOncoMatch-based alignment of lineage-matched cell lines to prostate carcinoma tumors. (**A**) Heatmap showing the conservation of the Tumor Checkpoint Reporter Set (TCRS) for each of 1045 tumors, from five different prostate carcinoma cohorts, in eight prostate carcinoma cell lines. The conservation was quantified by the enrichment of the TCRS on the cell lines' protein activity signature and is expressed by the Tumor Checkpoint Matching Score (TCMS) as $-\log_{10}(p\text{-value})$, Bonferroni's corrected. The bar graph to the right of the heatmap shows the Checkpoint Coverage Index (CCI), indicating the proportion (%) of the analyzed tumors whose checkpoint is significantly conserved in the cell lines ($p < 10^{-5}$, TCMS > 5). (**B**) Example enrichment plots for the TCRS of one tumor sample (TCGA-YL-A8S9-01) on the protein activity signatures of the DU145 and LNCaP cell lines. The *x*-axis represents 2448 transcriptional regulatory proteins, sorted from the one showing the lowest relative activity (left) to the one showing the highest relative activity (right) for the specified cell line. The vertical lines indicate the top 25 (red) and bottom 25 (blue) proteins most activated and inactivated, respectively, that are part of the TCRS for sample TCGA-YL-A8S9-01. The plots show strong enrichment of the TCRS in the LNCaP, but not the DU145, protein activity signatures.

The NEW ENGLAND JOURNAL of MEDICINE

Clinical and Translational Impact and Applications

In this report on the STORM clinical trial for multiple myeloma in which patients were treated with the XPO1 inhibitor selinexor, **DarwinHealth** identified a Linear Discriminant Analysis (LDA) classifier using **VIPER**-assessed activity of only four proteins (IRF3, ARL2BP, ZBTB17, and ATRX). The **DarwinOncoMarker** achieved highly significant predictability (AUC = 0.862) in identifying responders vs. non responders, as also confirmed in an independent cohort.



ORIGINAL ARTICLE

Oral Selinexor–Dexamethasone for Triple-Class Refractory Multiple Myeloma

A. Chari, D.T. Vogl, M. Gavriatopoulou, A.K. Nooka, A.J. Yee, C.A. Huff, P. Moreau, D. Dingli, C. Cole, S. Lonial, M. Dimopoulos, A.K. Stewart, J. Richter, R. Vij, S. Tuchman, M.S. Raab, K.C. Weisel, M. Delforge, R.F. Cornell, D. Kaminetzky, J.E. Hoffman, L.J. Costa, T.L. Parker, M. Levy, M. Schreder, N. Meuleman, L. Frenzel, M. Mohty, S. Choquet, G. Schiller, R.L. Comenzo, M. Engelhardt, T. Illmer, P. Vlummens, C. Doyen, T. Facon, L. Karlin, A. Perrot, K. Podar, M.G. Kauffman, S. Shacham, L. Li, S. Tang, C. Picklesimer, J.-R. Saint-Martin, M. Crochiere, H. Chang, S. Parekh, Y. Landesman, J. Shah, P.G. Richardson, and S. Jagannath

ABSTRACT

BACKGROUND

Selinexor, a selective inhibitor of nuclear export compound that blocks exportin 1 (XPO1) and forces nuclear accumulation and activation of tumor suppressor proteins, inhibits nuclear factor κB, and reduces oncoprotein messenger RNA translation, is a potential novel treatment for myeloma that is refractory to current therapeutic options.

METHODS

We administered oral selinexor (80 mg) plus dexamethasone (20 mg) twice weekly to patients with myeloma who had previous exposure to bortezomib, carfilzomib, lenalidomide, pomalidomide, daratumumab, and an alkylating agent and had disease refractory to at least one proteasome inhibitor, one immunomodulatory agent, and daratumumab (triple-class refractory). The primary end point was overall response, defined as a partial response or better, with response assessed by an independent review committee. Clinical benefit, defined as a minimal response or better, was a secondary end point.

RESULTS

A total of 122 patients in the United States and Europe were included in the modified intention-to-treat population (primary analysis), and 123 were included in the safety population. The median age was 65 years, and the median number of previous regimens was 7; a total of 53% of the patients had high-risk cytogenetic abnormalities. A partial response or better was observed in 26% of patients (95% confidence interval, 19 to 35), including two stringent complete responses; 39% of patients had a minimal response or better. The median duration of response was 4.4 months, median progression-free survival was 3.7 months, and median overall survival was 8.6 months. Fatigue, nausea, and decreased appetite were common and were typically grade 1 or 2 (grade 3 events were noted in up to 25% of patients, and no grade 4 events were reported). Thrombocytopenia occurred in 73% of the patients (grade 3 in 25% and grade 4 in 33%). Thrombocytopenia led to bleeding events of grade 3 or higher in 6 patients.

CONCLUSIONS

Selinexor–dexamethasone resulted in objective treatment responses in patients with myeloma refractory to currently available therapies. (Funded by Karyopharm Therapeutics; STORM ClinicalTrials.gov number, NCT02336815.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Jagannath at the Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, 1470 Madison Ave., 3rd Fl., New York, NY 10029, or at sundar.jagannath@mountsinai.org.

Drs. Richardson and Jagannath contributed equally to this article.

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pharm Therapeutics; Ms. Picklesimer, Mr. Saint-Martin, and Dr. Crochiere, being employed by and owning stock in Karyopharm Therapeutics; Dr. Parekh, receiving grant support from Karyopharm Therapeutics; Dr. Landesman, being employed by and owning stock in Karyopharm Therapeutics; Dr. Shah, being employed by Karyopharm Therapeutics; Dr. Richardson, receiving grant support and honoraria from Oncopeptides, Celgene, and Takeda, grant support from Bristol-Myers Squibb, and honoraria from Amgen, Janssen, and Karyopharm Therapeutics; Dr. Jagannath, receiving advisory board fees and consulting fees from Celgene, Bristol-Myers Squibb, Janssen Pharmaceuticals, and Merck. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

The authors' full names and academic degrees are as follows: Ajai Chari, M.D., Dan T. Vogl, M.D., Maria Gavriatopoulou, M.D., Ajay K. Nooka, M.D., Andrew J. Yee, M.D., Carol A. Huff, M.D., Philippe Moreau, M.D., David Dingli, M.D., Ph.D., Craig Cole, M.D., Sagar Lonial, M.D., Meletios Dimopoulos, M.D., A. Keith Stewart, M.B., Ch.B., Joshua Richter, M.D., Ravi Vij, M.D., Sascha Tuchman, M.D., Marc S. Raab, M.D., Katja C. Weisel, M.D., Michel Delforge, M.D., Ph.D., Robert F. Cornell, M.D., David Kaminetzky, M.D., James E. Hoffman, M.D., Luciano J. Costa, M.D., Ph.D., Terri L. Parker, M.D., Moshe Levy, M.D., Martin Schreder, M.D., Nathalie Meuleman, M.D., Ph.D., Laurent Frenzel, M.D., Ph.D., Mohamad Mohty, M.D., Ph.D., Sylvain Choquet, M.D., Gary Schiller, M.D., Raymond L. Comenzo, M.D., Monika Engelhardt, M.D., Thomas Illmer, M.D., Philip Vlummens, M.D., Chantal Doyen, M.D., Thierry Facon, M.D., Lionel Karlin, M.D., Aurore Perrot, M.D., Ph.D., Klaus Podar, M.D., Ph.D., Michael G. Kauffman, M.D., Ph.D., Sharon Shacham, Ph.D., Lingling Li, Ph.D., Shijie Tang, Ph.D., Carla Picklesimer, B.S., Jean-Richard Saint-Martin, B.S., Marsha Crochiere, Ph.D., Hua Chang, Ph.D., Samir Parekh, M.B., B.S., Yosef Landesman, Ph.D., Jatin Shah, M.D., Paul G. Richardson, M.D., and Sundar Jagannath, M.B., B.S.

The authors' affiliations are as follows: the Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai (A.C., S.P., S.J.), and New York University Langone Medical Center (D.K.) — both in New York; the Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia (D.T.V.); the School of Medicine, National and Kapodistrian University of Athens, Athens (M.G., M. Dimopoulos); the Winship Cancer Institute, Emory University, Atlanta (A.K.N., S.L.); Massachusetts General Hospital Cancer Center (A.J.Y.), Tufts Medical Center (R.L.C.), and the Dana–Farber Cancer Institute (P.G.R.), Boston, and Karyopharm Therapeutics, Newton (M.G.K., S.S., L.L., S. Tang, C.P., J.-R.S.-M., M.C., H.C., Y.L., J.S.) — all in Massachusetts; Johns Hopkins University, Baltimore (C.A.H.); the University of Nantes, Nantes (P.M.), Hôpital Necker (L.F.), Hôpital Saint-Antoine (M.M.), and La Pitié–Salpêtrière Hospital (S.C.), Paris, University Hospital, Lille (T.F.), Centre Hospitalier Lyon Sud, Pierre-Benite (L.K.), and Centre Hospitalo-Universitaire Vandoeuvre-lès-Nancy, Nancy (A.P.) — all in France; the Mayo Clinic, Rochester, MN (D.D.); the University of Michigan, Ann Arbor (C.C.); the Mayo Clinic of Arizona, Phoenix (A.K.S.); Hackensack University Medical Center, Hackensack, NJ (J.R.); Washington University School of Medicine, St. Louis (R.V.); Lineberger Comprehensive Cancer Center at University of North Carolina–Chapel Hill, Chapel Hill (S. Tuchman); the University of Heidelberg, Heidelberg (M.S.R.), University Medical Center Hamburg-Eppendorf, Hamburg (K.C.W.), the University of Tübingen, Tübingen (K.C.W.), University Hospital Würzburg, Würzburg (M.S.), the University of Freiburg, Freiburg (M.E.), and Gemeinschaftspraxis Hämatologie-Onkologie, Dresden (T.I.) — all in Germany; the University of Leuven, Leuven (M. Delforge), Institut Jules Bordet, Université Libre de Bruxelles, Brussels (N.M.), University Hospital Ghent, Ghent (P.V.), and Centre Hospitalier Universitaire Université Catholique de Louvain Namur, Yvoir (C.D.) — all in Belgium; Vanderbilt University Medical Center, Nashville (R.F.C.); Sylvester Cancer Center, University of Miami, Miami (J.E.H.); the University of Alabama at Birmingham, Birmingham (L.J.C.); Yale School of Medicine, New Haven, CT (T.L.P.); Baylor University Medical Center, Dallas (M.L.); the David Geffen School of Medicine at the University of California, Los Angeles, Los Angeles (G.S.); and University Hospital Krems, Karl Landsteiner University of Health Sciences, Krems an der Donau, Austria (K.P.).

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higher were exclusion criteria. A full list of inclusion and exclusion criteria is provided in Table S1 in the Supplementary Appendix. Written informed consent was obtained from all patients before enrollment.

TREATMENT

Oral selinexor (80 mg) in combination with dexamethasone (20 mg) was administered on days 1 and 3, weekly, in 4-week cycles until disease progression, death, or discontinuation. A dose-modification protocol was used for the management of adverse events (Tables S2 and S3 in the Supplementary Appendix). All patients were required to receive 8 mg of ondansetron (or equivalent) before the first dose of study drug and two or three times daily as needed. Other antiemetics (olanzapine and neurokinin-1 receptor antagonists) were permitted for patients with unacceptable side effects to ondansetron (or its equivalent) or with persistent nausea. Supportive measures were provided at the discretion of the investigator and may have included intravenous fluids, hematopoietic growth factors, transfusions, appetite stimulants (olanzapine and megestrol acetate), or a combination of these.

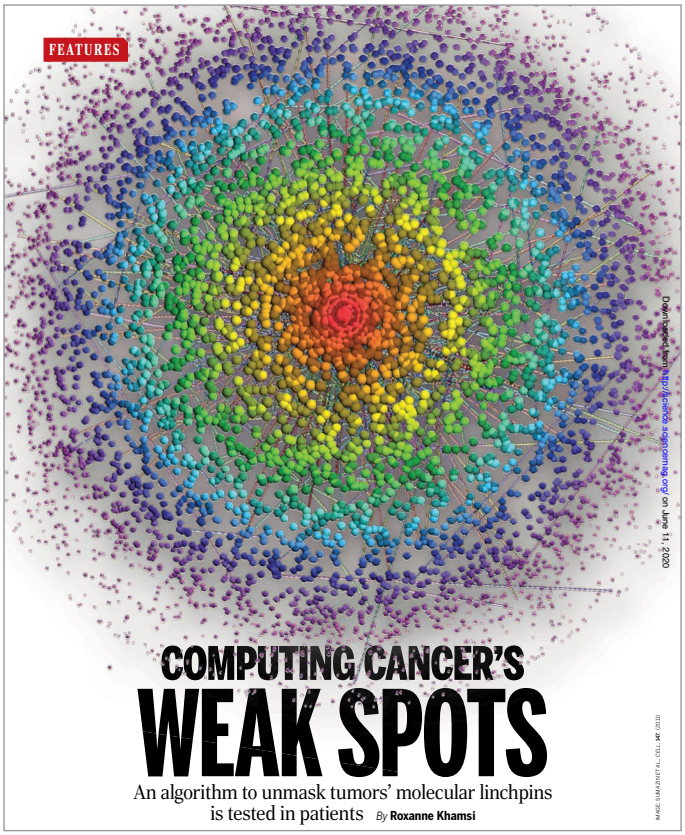
and 12-lead electrocardiography. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.[23]

PHARMACODYNAMICS AND RESPONSE PREDICTOR

Methods regarding measurement of XPO1 mRNA induction and immunohistochemical analysis of glucocorticoid receptor induction are included in the Supplementary Appendix. A predictive biomarker of response to selinexor was sought in patients with myeloma with the use of the VIPER (Virtual Inference of Protein-activity by Enriched Regulon analysis) algorithm, which can transform gene-expression profiles from tumor samples into accurate predictions of protein activity for approximately 6000 regulatory proteins (DarwinHealth) (see the Supplementary Appendix).[24] RNA levels in CD138+ cells that were isolated from the pretreatment bone marrow aspirate of patients enrolled in the STORM Part 2 study were used for this analysis.

STATISTICAL ANALYSIS

The sample size was based on assumptions for penta-exposed, triple-class refractory myeloma



FEATURES

COMPUTING CANCER'S WEAK SPOTS

An algorithm to unmask tumors' molecular linchpins is tested in patients By **Roxanne Khamsi**

IMAGE: SAMANTHA L. COLE, UCI; (2018)

In 2016, doctors invited Eileen Kapotes to join a clinical trial for a drug that had never been used for her disease. Kapotes, a first grade teacher in her 50s, was fighting an aggressive breast cancer that had spread through her body. She had endured grueling treatments over the previous 4 years, including whole-brain radiation therapy. She had also been taking the breast cancer medication Herceptin, but her tumors were still growing. Now, she had a chance to try something radically different: a drug called ruxolitinib, originally designed to treat cancers affecting the blood and bone marrow.

Kapotes's oncologist, Amy Tiersten at Mount Sinai Hospital, was stunned by how well her patient responded to the new drug. It kept her cancer at bay and she had almost no side effects. "I was amazed," Tiersten says.

The ruxolitinib trial was the product of a decadelong quest by Andrea Califano, a systems biologist at Columbia University. Using sophisticated computing, he models the molecular networks that sustain cancer cells and pinpoints proteins called transcription factors that act as linchpins, controlling the behavior of many genes inside a cell. Califano collaborated with cell biologist José Silva, then also at Columbia, to analyze breast cancer samples in a repository of tissues from other patients who had become resistant to Herceptin. Findings of the analysis suggested a transcription factor called STAT3 plays a critical role in those cancers. And ruxolitinib was known to inhibit STAT3.

Other researchers have focused on identifying genetic mutations that drive the disease in an individual patient. Doing so, the thinking goes, can help identify the best drug for each patient. But because of the diversity of cancer-causing mutations across the population, an arsenal of tens of thousands of drugs might be needed to treat everyone.

Califano's approach, by contrast, is a twist on that idea. He has focused instead on identifying a few transcription factors that act as bottlenecks (see graphic, p. 1176). Target those master regulators, as Califano calls them, and you will stop cancer in its tracks, no matter what mutation initially caused it. Oncologists would still need to analyze each patient's mutations to figure out which regulators are at play in their particular cancer, but instead of tens of thousands of drugs, Califano says, they may only need dozens. It's a depersonalized approach to personalized medicine.

A visualization of 7000 interacting genes in a brain cancer patient shows the handful with the largest number of interactions (red)—which makes them tempting targets for therapies.

The strategy builds on Califano's computational training as a physicist. "We've built algorithms that can reverse engineer the logic of each different tumor so that we know the targets" for drugs, he says. His algorithms are a prime example of systems biology—which uses complicated math to model intricate biological systems, such as gene interactions. It's a field that has generated tremendous interest, but little real-world clinical success.

In 2015, Califano co-founded a company called DarwinHealth that uses his algorithms to guide doctors by identifying the key transcription factors in a patient's tumor and suggesting drugs to target them. His work has earned praise from other researchers, although some note the approach is only in early stages of human testing, and its clinical usefulness remains uncertain. Ed Liu, president and CEO of the Jackson Laboratory, a nonprofit biomedical institute Califano has collaborated with, is optimistic the method will ultimately pay off. "As we develop more and more precise ways to attack those nodes, then the more useful his algorithms will be."

> **"You have more potential combinations of cancerous mutations than atoms in the universe."**
> **Andrea Califano**, Columbia University

Califano's approach is about to get its largest test yet. Columbia has allocated $15 million for a trial of 3000 cancer patients within its hospitals over the next 3 years, using DarwinHealth algorithms to analyze each patient's cancer and recommend treatments. "This is probably one of the most exciting moments in my research," Califano says, "because finally we're able to apply this methodology on a scale that is large enough to be able to really learn something in terms of the response of the patient."

IN FALL OF 1958, a young scientist named François Jacob went to his colleague Jacques Monod at the Pasteur Institute in Paris with a hypothesis about how genetic mechanisms might control cell behavior. Both men had renegade tendencies: Jacob had fought Nazis—and been injured—on behalf of the exiled French government in World War II, and Monod, an accomplished rock climber, had taken part in the guerilla activities of the French Resistance. Over the next few years, the pair worked together, and they were the first to demonstrate the idea of genetic circuits. The work ultimately won them a share of the 1965 Nobel Prize in Physiology or Medicine.

In experiments with *Escherichia coli*, Jacob and Monod showed that the gene networks in those bacteria can alter the production of certain enzymes depending on the type of food available. When the sugar lactose was abundant, the bacteria turned on genes that code for the enzymes to metabolize it. But with access only to glucose, a different sugar, the microbes shut down those genes. It was a pioneering demonstration that the activity of individual genes could be either boosted or repressed.

Experiments in later decades helped explain how the cell machinery exerts that control. One key player is transcription factors, proteins that boost or inhibit the activity of other genes. The gene-regulating network of a single cell is far more elaborate than Jacob and Monod had the tools to uncover. The human genome contains 20,000 genes, and an estimated 1500 of those produce transcription factors. That system creates a complex web of on and off switches.

Califano thought that if he could identify the key switches in cancer, he might be able to shut down the catastrophic genetic changes that drive its growth. But after he finished his training as a physicist in 1986, IBM recruited him to spearhead projects in computer vision and artificial intelligence. The building codes at the IBM facility prevented Califano from having an experimental lab to pursue his interests in biology. He left in 2000 and landed at Columbia in 2003. He started to write code to solve the riddle of cancer on the day he arrived.

Nowadays, the data underlying his algorithms come from a method called RNA sequencing (RNA-seq). The method gauges gene activity within cells by sequencing RNA molecules, which act as a proxy for which genes are turned on and off. Algorithms crunch the massive amount of RNA-seq data to reveal which genes are overactive or underactive in cancer compared with healthy tissue. The algorithms then use complex equations to infer patterns of gene interactions and zero in on the transcription factors with the largest influence.

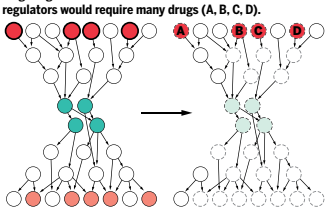
The search for key drivers of cancer isn't easy. Consider a 2018 analysis of more than 9000 samples that reported almost 1.5 million mutations. Genes influence one another in intricate webs and feedback

Hitting cancer's choke points

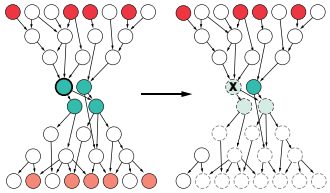
Shutting down a cancer cell's malfunctioning gene network is a tall order if you target mutations too far upstream in the network. But disabling a transcription factor that acts as a master regulator in the cell's genetic circuitry can cause its demise even with just one drug.

○ Gene ◌ Corrected gene behavior ● Master regulator
● Initial mutation ● Malfunctioning gene

Targeting all mutations that can activate cancer's master regulators would require many drugs (A, B, C, D).



A single drug (X) that targets a master regulator can have the same effect.



hunt. It has contracted DarwinHealth to systematically search the pharmaceutical giant's library of compounds for other compounds that might target master regulators.

Additional support for the DarwinHealth approach comes from a recent study by Samir Parekh at the Icahn School of Medicine at Mount Sinai and a team of international collaborators, who recently completed a clinical trial to test a combination of two drugs, dexamethasone and selinexor, for multiple myeloma. The combination only worked in about one-quarter of patients, reducing levels of a myeloma protein in their blood. In a retrospective analysis, the DarwinHealth tools predicted which patients would respond. By assessing RNA-seq data from 12 patients, the tools identified four of the five patients who benefited from the drugs and six of the seven who did not, the researchers reported last year in *The New England Journal of Medicine*.

Morgan Craig, who uses computational approaches to identify new drugs at the University of Montreal, says efforts to understand molecular networks in cancer have the potential to improve personalized medicine. Algorithms like those used by DarwinHealth "may not take over clinical approaches right away," Craig explains. "But it's definitely a step toward trying to do this target identification in a more systematic way."

DARWINHEALTH DOESN'T RUN clinical trials, but for the past 3 years, Califano's lab has tested the company's algorithms in experiments at Columbia. The researchers analyzed RNA-seq data from biopsy samples from more than 100 cancer patients to identify master regulators and suggest drugs that might not normally be considered (much as DarwinHealth's commercial service does). In a few dozen cases, the researchers later tested the drug in mice with a grafted version of a patient's tumor to confirm the drug affected the master regulators as predicted. For five of those patients, doctors felt bold enough to try the algorithm's suggested drug. Each patient had late-stage

Califano hopes to build on those anecdotal results with the formal clinical trial, now underway at Columbia. The Oncotarget and Oncotreat tests from DarwinHealth will be used with 3000 patients in the Columbia system. Ultimately, the drugs they receive will be chosen by a board of doctors on the basis of readouts either from mutations detected by traditional sequencing or from the VIPER-based algorithm. DarwinHealth will receive no money for the tests to avoid clinical studies will all focus on gastrointestinal tumors, particularly gastric and esophageal cancer," Zhang says.

As the DarwinHealth approach goes into more clinical testing sites, more patients like Kapotes will receive drugs never intended to fight their particular cancers. For some people, like her, it might buy precious time. For more than 2 years after she enrolled in the ruxolitinib trial, Kapotes's cancer remained stable. When scans eventually showed her tumor had

Andrea Califano (right) and Gideon Bosker (left) founded DarwinHealth to apply systems biology to cancer.

CANCER DISCOVERY

Clinical and Translational Impact and Applications

In this 7-year clinical trial evaluating multiple advanced cancers, tumors resected from 130 patients–across 18 heterogeneous, highly aggressive malignancies that had progressed on multiple lines of treatment–were first transplanted in immunocompromised mice and then treated with drugs predicted by **DarwinOncoTreat** or **DarwinOncoTarget**. Based on 35 predicted drug arms (22 by **OncoTreat**, 21 by **OncoTarget**, with 8 predicted by both methods) and tyrosine kinase inhibitors (negative control arms), the trial showed a DCR (Disease Control Rate) = 68% and DCR = 91% compared to a DCR = 0% for drugs on the control arms.

RESEARCH ARTICLE

A Transcriptome-Based Precision Oncology Platform for Patient–Therapy Alignment in a Diverse Set of Treatment-Resistant Malignancies

Prabhjot S. Mundi[1,2], Filemon S. Dela Cruz[3], Adina Grunn[1], Daniel Diolaiti[3], Audrey Mauguen[4], Allison R. Rainey[3], Kristina Guillan[3], Armaan Siddiquee[3], Daoqi You[3], Ronald Realubit[1], Charles Karan[1,2], Michael V. Ortiz[3], Eugene F. Douglass Jr[1], Melissa Accordino[2,5], Suzanne Mistretta[2], Frances Brogan[2], Jeffrey N. Bruce[2,6], Cristina I. Caescu[1], Richard D. Carvajal[2,5], Katherine D. Crew[2,5], Guarionex Decastro[2,7], Mark Heaney[2,5], Brian S. Henick[2,5], Dawn L. Hershman[2,5,8], June Y. Hou[9], Fabio M. Iwamoto[2,10], Joseph G. Jurcic[2,5], Ravi P. Kiran[2,11], Michael D. Kluger[2,11], Teri Kreisl[10], Nicole Lamanna[2,5], Andrew B. Lassman[2,10], Emerson A. Lim[2,5], Gulam A. Manji[2,5], Guy M. McKhann[2,6], James M. McKiernan[2,7], Alfred I. Neugut[2,5,8], Kenneth P. Olive[2,5], Todd Rosenblat[2,5], Gary K. Schwartz[2,5], Catherine A. Shu[2,5], Michael B. Sisti[2,6,12,13], Ana Tergas[9], Reena M. Vattakalam[2,9], Mary Welch[2,10], Sven Wenske[2,7], Jason D. Wright[2,9], Peter Canoll[2,14], Hanina Hibshoosh[2,14], Kevin Kalinsky[2,15], Mahalaxmi Aburi[1], Peter A. Sims[1,16], Mariano J. Alvarez[1,17], Andrew L. Kung[3], and Andrea Califano[1,2,5,16,18,19]

ABSTRACT Predicting *in vivo* response to antineoplastics remains an elusive challenge. We performed a first-of-kind evaluation of two transcriptome-based precision cancer medicine methodologies to predict tumor sensitivity to a comprehensive repertoire of clinically relevant oncology drugs, whose mechanism of action we experimentally assessed in cognate cell lines. We enrolled patients with histologically distinct, poor-prognosis malignancies who had progressed on multiple therapies, and developed low-passage, patient-derived xenograft models that were used to validate 35 patient-specific drug predictions. Both OncoTarget, which identifies high-affinity inhibitors of individual master regulator (MR) proteins, and OncoTreat, which identifies drugs that invert the transcriptional activity of hyperconnected MR modules, produced highly significant 30-day disease control rates (68% and 91%, respectively). Moreover, of 18 OncoTreat-predicted drugs, 15 induced the predicted MR-module activity inversion *in vivo*. Predicted drugs significantly outperformed antineoplastic drugs selected as unpredicted controls, suggesting these methods may substantively complement existing precision cancer medicine approaches, as also illustrated by a case study.

SIGNIFICANCE: Complementary precision cancer medicine paradigms are needed to broaden the clinical benefit realized through genetic profiling and immunotherapy. In this first-in-class application, we introduce two transcriptome-based tumor-agnostic systems biology tools to predict drug response *in vivo*. OncoTarget and OncoTreat are scalable for the design of basket and umbrella clinical trials.

INTRODUCTION

A major goal of precision cancer medicine (PCM) is to improve clinical outcomes by leveraging the molecular-level properties of a tumor—as encoded by mutational, gene expression, epigenetic modification, and proteomic profiles—to accurately predict sensitivity to candidate therapeutic agents. Application of PCM principles may help generate responder-enriched cohorts for clinical trials when predictions are conserved across a substantial fraction of patients (1, 2), and even help prioritize personalized treatments on an individual patient basis.

Systematic application of the current PCM paradigm is largely predicated on two complementary approaches. The first one (oncogene addiction) aims to identify targeted therapies based on the presence of activating genetic alterations in druggable oncoproteins (3); the second (immunotherapy) is based on the discovery that specific tumor-initiated immunosuppressive programs can be abrogated by pharmacologic targeting of immune checkpoints or by sensitizing the immune system to tumor antigens (4).

Despite the remarkable clinical success of these approaches within specific tumor subtypes (5), many tumors may lack actionable genetic alterations, fail to respond to therapy, or develop drug resistance, suggesting an acute need for complementary approaches targeting nononcogene tumor dependencies (6). In particular, despite its critical role in tumor subtype stratification, use of transcriptome-based approaches in precision medicine has lagged.

We and others have shown that, within each tumor histology, cancer cells adopt only a relatively limited, discrete, and remarkably stable repertoire of molecularly distinct transcriptional states (7). These states are mechanistically

[1]Department of Systems Biology, Columbia University Irving Medical Center, New York, New York. [2]Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center, New York, New York. [3]Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, New York. [4]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, New York. [5]Department of Medicine, Columbia University Irving Medical Center, New York, New York. [6]Department of Neurological Surgery, Columbia University Irving Medical Center, New York, New York. [7]Department of Urology, Columbia University Irving Medical Center, New York, New York. [8]Department of Epidemiology, Columbia University Mailman School of Public Health, New York, New York. [9]Department of Obstetrics and Gynecology, Columbia University Irving Medical Center, New York, New York. [10]Department of Neurology, Columbia University Irving Medical Center, New York, New York, [11]Department of Surgery, Columbia University Irving Medical Center, New York, New York. [12]Department of Urology, Columbia University Irving Medical Center, New York, New York. [13]J.P. Sulzberger Columbia Genome Center, Columbia University Irving Medical Center, New York, New York. [14]Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, New York. [15]Winship Cancer Institute of Emory University and Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, Georgia. [16]Department of Biochemistry and Molecular Biophysics, Columbia University Irving Medical Center, New York, New York. [17]DarwinHealth Inc., New York, New York. [18]Department of Biomedical Informatics, Columbia University Irving Medical Center, New York, New York. [19]J.P. Sulzberger Columbia Genome Center, Columbia University Irving Medical Center, New York, New York.

Note: P.S. Mundi and F.S. Dela Cruz contributed equivalently to this article.

Corresponding Authors: Andrea Califano, Department of Systems Biology, Columbia University, 1130 St. Nicholas Avenue, New York, NY 10032. Phone: 212-851-5140; E-mail: ac2248@columbia.edu; Mariano J. Alvarez, 3960 Broadway, New York, NY 10032. Phone: 646-661-5604; E-mail: malvarez@darwinhealth.com; and Andrew L. Kung, 1275 York Avenue, New York, NY 10065. Phone: 212-639-5957; E-mail: kunga@mskcc.org

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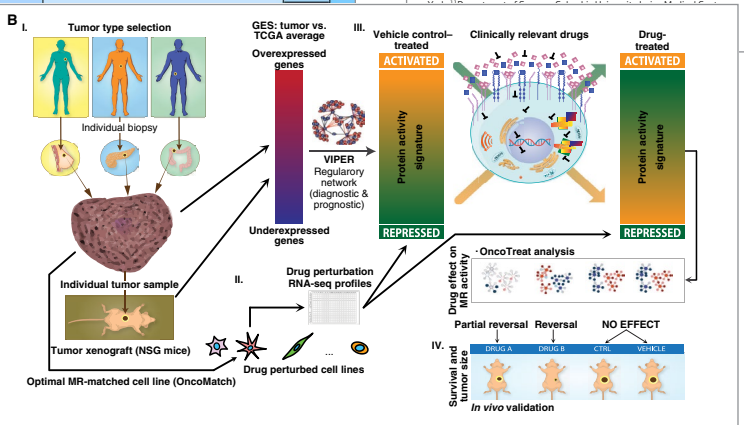

Figure 1. N-of-1 study overview. **A,** Clinical characteristics, prior systemic treatment, and tumor genomic profiling (if available) for the seven subjects. **B,** Study conceptual diagram. **I,** Adults with advanced solid tumors with progression or intolerance to standard treatments are enrolled. Fresh biopsy tissue is partitioned for clinical pathology review, RNA-seq, and xenografting into immunodeficient mice (PDX). Engrafted PDX tumors are also profiled by RNA-seq and VIPER to confirm fidelity to the patient-derived tumor (OncoMatch; see Methods). **II,** High-throughput drug screens have been completed in cognate cell lines with high fidelity to distinct cohorts of patient tumors based on recapitulation of MR protein activity (Bonferroni $P < 10^{-10}$ by OncoMatch), collectively comprising the PanACEA database. Cells were perturbed at sublethal drug concentrations, and VIPER analysis of postperturbation RNA-seq allows for *de novo* mechanism inference for each drug in each cellular context. **III,** VIPER analysis of the patient tumor identifies top MR proteins and drugs are predicted by two methods. First, individual activated druggable MR proteins, e.g., protein kinases and epigenetic regulatory enzymes, are identified (Bonferroni $P < 10^{-5}$ by OncoTarget). Second, using the best matched cell line(s) in PanACEA, drugs are ranked based on their inverting effect on the top MR proteins, i.e., TCM-inverting drugs (Bonferroni $P < 10^{-5}$ by OncoTreat). **IV,** Up to six predicted drugs are selected for experimental validation, based on OncoTarget or OncoTreat *P*-value and a number of practical selection criteria. Mice from early PDX passages (usually P1) are randomized into candidate drug arms, negative control drug arms, and a vehicle control arm.

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bjh research paper

Fimepinostat (CUDC-907) in patients with relapsed/refractory diffuse large B cell and high-grade B-cell lymphoma: report of a phase 2 trial and exploratory biomarker analyses

Daniel J. Landsburg,[1] Stefan K. Barta,[1,2] Radhakrishnan Ramchandren,[3,4] Connie Batlevi,[5] Swaminathan Iyer,[6,7] Kevin Kelly,[8] Ivana N. Micallef,[9] Sonali M. Smith,[10] Don A. Stevens,[11] Mariano Alvarez,[12] Andrea Califano,[12] Yao Shen,[12] Gideon Bosker,[12] Jefferson Parker,[13] Raul Soikes,[13] Elizabeth Martinez,[13] Reinhard von Roemeling,[13] Robert E. Martell[13] and Yasuhiro Oki[6,14]

[1]*University of Pennsylvania,* [2]*Fox Chase Cancer Center, Philadelphia, PA,* [3]*University of Tennessee Knoxville, Knoxville, TN,* [4]*Karmanos Cancer Institute, Detroit, MI,* [5]*Memorial Sloan Kettering Cancer Center, New York, NY,* [6]*MD Anderson Cancer Center,* [7]*Houston Methodist Hospital, Houston, TX,* [8]*University of Southern California, Los Angeles, CA,* [9]*Mayo Clinic, Rochester, MN,* [10]*University of Chicago, Chicago, IL,* [11]*Norton Cancer Institute, Louisville, KY,* [12]*DarwinHealth, Inc, New York, NY,* [13]*Curis, Inc, Lexington, MA, and* [14]*Genentech, San Francisco, CA, USA*

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Correspondence: Daniel J. Landsburg, 3400 Civic Center Blvd, #12-182, Philadelphia, PA 19104, USA.
E-mail: daniel.landsburg@pennmedicine.upenn.edu

Summary

Fimepinostat (CUDC-907), a first-in-class oral small-molecule inhibitor of histone deacetylase and phosphatidylinositol 3-kinase, demonstrated efficacy in a phase 1 study of patients with relapsed/refractory (R/R) diffuse large and high-grade B-cell lymphomas (DLBCL/HGBL), particularly those with increased MYC protein expression and/or *MYC* gene rearrangement/copy number gain (MYC-altered disease). Therefore, a phase 2 study of fimepinostat was conducted in this patient population with 66 eligible patients treated. The primary end-point of overall response (OR) rate for patients with MYC-IHC ≥40% ($n = 46$) was 15%. Subsequently, exploratory pooled analyses were performed including patients treated on both the phase 1 and 2 studies based upon the presence of MYC-altered disease as well as a biomarker identified by Virtual Inference of Protein activity by Enriched Regulon analysis (VIPER). For these patients with MYC-altered disease ($n = 63$), the overall response (OR) rate was 22% with seven responding patients remaining on treatment for approximately two years or longer, and VIPER yielded a three-protein biomarker classification with positive and negative predictive values of ≥85%. Prolonged durations of response were achieved by patients with MYC-altered R/R DLBCL/HGBL treated with single-agent fimepinostat. Combination therapies and/or biomarker-based patient selection strategies may lead to higher response rates in future clinical trials.

Keywords: diffuse large B-cell lymphoma, MYC, histone deacetylase inhibitor, phosphatidylinositol 3-kinase inhibitor, biomarker.

included IHC staining of MYC (rabbit clone Y69) and BCL2 (mouse clone 124) as well as FISH with *MYC* (8q24) and *BCL6* (3q27) break-apart probes and *BCL2* [t(14;18)] fusion probe performed by NeoGenomics Laboratories, Inc (Fort Myers, FL, USA), with positive cut-off values for *MYC* rearrangement (>10%), *MYC* copy number gain (>20%), *BCL2* rearrangement (≥0·5%) and *BCL6* rearrangement (>10%) as defined per laboratory standard. Cell of origin was defined as per Hans algorithm[21] by local testing.

Patients were prospectively classified into three categories based upon the presence or absence of MYC alterations. Group A was characterized by the presence of MYC-R or MYC-CN without MYC-IHC, Group B as MYC-IHC with or without MYC-R and/or MYC-CN and Group C as no MYC alterations identified by central testing or central testing unable to be performed due to lack of adequate tissue.

The primary objective was to determine the OR rate for Group B patients as per central radiographic review. Key secondary objectives were to determine the OR rate by local radiographic review, complete response (CR) rate, progression-free survival (PFS), overall survival (OS), disease control (DC) rate and duration of response (DOR) for Group B patients, determine the OR rate for Groups A and C and to evaluate the incidence and severity of adverse events (AE). Radiographic responses to treatment were made according to the Revised Response Criteria for Malignant Lymphoma.[22] Disease progression could also be defined by the investigator after consideration of clinical or laboratory features in the absence of diagnostic imaging. Survival times were estimated via the Kaplan–Meier method and 95% confidence intervals (CI) calculated via the binomial exact method. All statistical analyses were performed using Stata version 13 (StataCorp, College Station, TX, USA). A clinically meaningful OR rate was determined to be 30% with a sample size of 100 patients enrolled in Group B. An interim analysis was planned to occur when 50 patients had been enrolled in Group B, of which at least 25 patients were considered evaluable per protocol, with the lower bound of the 95% CI for OR to exceed 10% in evaluable Group B patients for the study to continue enrolment.

For the exploratory analysis of patients with MYC-altered disease, included patients were those from the evaluable population of the phase 1 protocol and the ITT population of the phase 2 protocols with MYC alterations as defined by central testing, or local testing in cases for which central testing for all of the following MYC alterations was not performed.

reproducible, and this methodology (DarwinOncoTarget algorithm) has been approved by the NYS Department of Health CLIA/CLEP Validation Unit as an offering in the category of "Molecular and Cellular Tumour Markers for Oncology"[24] and shown to be effective for biomarker discovery.[25] The activity of 6 213 regulatory proteins annotated as Transcription Factors (GO:0003700, or GO:0004677 and GO:0030528 or GO:0045449) or co-Transcription Factors (GO:0003712 or GO:0030528 or GO:0045449) or signaling proteins (GO:0007165 and GO:0005622 or GO:0005886) in the Gene Ontology[26] was inferred by metaVIPER,[27] using transcriptional regulatory networks (interactomes) inferred by analysis of a DLBCL and an acute myeloid leukemia (AML) cohorts using the ARACNe algorithm.[28] MetaVIPER is an extension of the VIPER algorithm supporting integration of multiple regulatory networks. A fimepinostat-sensitivity classifier was generated by training a Neural Network[29] using the top $k = 1, \ldots, 10$ most differentially active proteins between responders and non-responders samples. The data set is available in Gene Expression Omnibus accession GSE171806.

Results

Seventy patients were enrolled on the phase 2 protocol, with four patients excluded from analysis due to never having been dosed with fimepinostat (2) or lacking confirmation of receipt of 2–4 lines of prior therapy (2), resulting in 66 patients included in the ITT population.

Baseline characteristics of the ITT population are described in Table I. Response and survival outcomes are described in Table II and are based upon local radiographic review. The OR rate for Group B patients ($n = 46$) was 15% (95% CI 6–29%) and the OR rate for all patients ($n = 66$) was 12% (95% CI 5–22%). Of note, seven out of eight responding patients were in Group B. Additionally, two responding patients proceeded to autologous stem cell transplantation. For all patients, the median time to response was 2·6 months.

Treatment-emergent adverse events (TEAE) occurring per patient by highest grade experienced with a frequency of ≥10% are listed in Table III. Three patients experienced a grade 5 TEAE: Guillain–Barré syndrome deemed unlikely related to treatment in one patient, sepsis deemed not related to treatment in one patient and tracheal obstruction deemed not related to treatment in one patient. One patient discontinued treatment due to grade 2 vomiting deemed related to

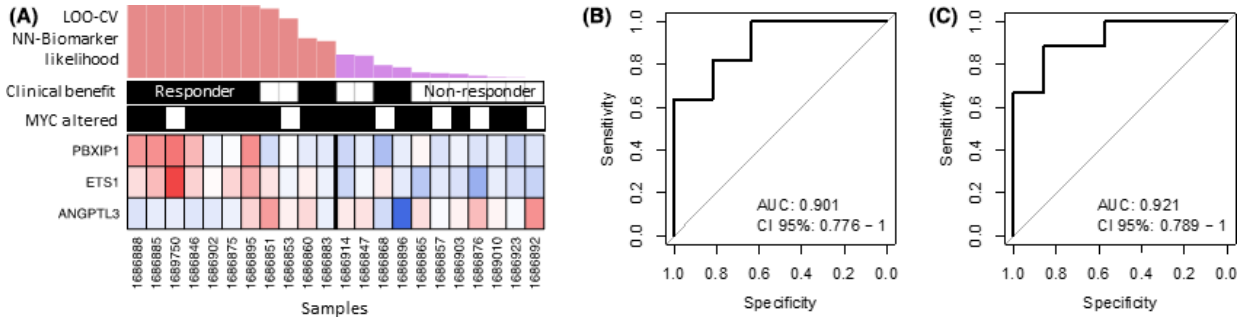

Fig 2. Leave-one-outcross-validation (LOO-CV) analysis for fimepinostat response biomarkers. (A) Heatmap showing the Virtual Inference of Protein-activity by Enriched Regulon analysis (VIPER)-inferred activity for the three fimepinostat response Master Regulator (MR) proteins used by the biomarkers (rows) for all samples. The clinical samples included in the analysis (columns) were rank-sorted based on the predicted likelihood of response by the NN-biomarker (barplot above the heatmap), estimated using LOO-CV. Patients that responded to fimepinostat [complete response (CR) and partial response (PR)] and patients that did not respond for fimepinostat [progressive disease (PD)] are shown in black and white, respectively (Clinical benefit row). Patients with MYC-altered disease are indicated in black in the MYC-altered row. (B, C) receiver operating characteristic (ROC) analysis for the LOO-CV performed on all samples ($n = 22$; B) and only on the MYC-altered samples ($n = 16$; C). Area under the ROC curve (AUC) and its 95% confidence interval (CI) is shown inside each plot.

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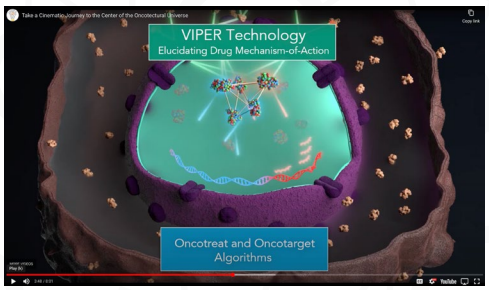

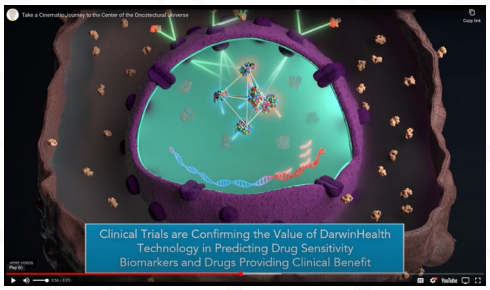

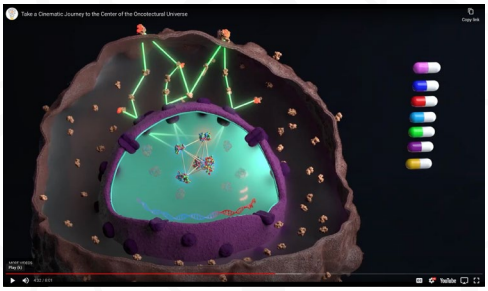

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