Please cite this article in press as: Irmisch et al., The Tumor Profiler Study: integrated, multi-omic, functional tumor profiling for clinical decision support, Cancer Cell (2021), https://doi.org/10.1016/j.ccell.2021.01.004

Cancer Cell

CellPress

Commentary

The Tumor Profiler Study: integrated, multi-omic, functional tumor profiling for clinical decision support

Anja Irmisch,[1,20] Ximena Bonilla,[2,3,4,20] Stéphane Chevrier,[5,20] Kjong-Van Lehmann,[2,3,4,20] Franziska Singer,[3,6,20] Nora C. Toussaint,[3,6,20] Cinzia Esposito,[7,20] Julien Mena,[8,20] Emanuela S. Milani,[9,20] Ruben Casanova,[5,20] Daniel J. Stekhoven,[3,6,20] Rebekka Wegmann,[8,20] Francis Jacob,[10,20] Bettina Sobottka,[11,20] Sandra Goetze,[9,20] Jack Kuipers,[3,12,20] Jacobo Sarabia del Castillo,[7,20] Michael Prummer,[3,6] Mustafa A. Tuncel,[3,12] Ulrike Menzel,[12] Andrea Jacobs,[5] Stefanie Engler,[5] Sujana Sivapatham,[5] Anja L. Frei,[11] Gabriele Gut,[7] Joanna Ficek,[2,3,4] Nicola Miglino,[13] Tumor Profiler Consortium, Rudolf Aebersold,[8,21] Marina Bacac,[14,21] Niko Beerenwinkel,[3,12,21] Christian Beisel,[12,21] Bernd Bodenmiller,[5,15,21] Reinhard Dummer,[1,21] Viola Heinzelmann-Schwarz,[10,16,21] Viktor H. Koelzer,[11,21] Markus G. Manz,[13,21] Holger Moch,[11,21] Lucas Pelkmans,[7,21] Berend Snijder,[3,8,21] Alexandre P.A. Theocharides,[13,21] Markus Tolnay,[17,21] Andreas Wicki,[13,18,21] Bernd Wollscheid,[9,21] Gunnar Rätsch,[2,3,4,19,21,*] and Mitchell P. Levesque[1,21,*]

[1]University Hospital Zurich, Department of Dermatology, University of Zurich, Gloriastrasse 31, 8091 Zurich, Switzerland
[2]ETH Zurich, Department of Computer Science, Institute of Machine Learning, Universitätstrasse 6, 8092 Zurich, Switzerland
[3]SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland
[4]University Hospital Zurich, Biomedical Informatics, Schmelzbergstrasse 26, 8006 Zurich, Switzerland
[5]University of Zurich, Department of Quantitative Biomedicine, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[6]ETH Zurich, NEXUS Personalized Health Technologies, John-von-Neumann-Weg 9, 8093 Zurich, Switzerland
[7]University of Zurich, Department of Molecular Life Sciences, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[8]ETH Zurich, Department of Biology, Institute of Molecular Systems Biology, Otto-Stern-Weg 3, 8093 Zurich, Switzerland
[9]ETH Zurich, Department of Health Sciences and Technology, Otto-Stern-Weg 3, 8093 Zurich, Switzerland
[10]University Hospital Basel and University of Basel, Department of Biomedicine, Hebelstrasse 20, 4031 Basel, Switzerland
[11]University Hospital Zurich, Department of Pathology and Molecular Pathology, Schmelzbergstrasse 12, 8091 Zurich, Switzerland
[12]ETH Zurich, Department of Biosystems Science and Engineering, Mattenstrasse 26, 4058 Basel, Switzerland
[13]University Hospital Zurich, Department of Medical Oncology and Hematology, Rämistrasse 100, 8091 Zurich, Switzerland
[14]Roche Pharmaceutical Research and Early Development, Roche Innovation Center Zurich, Wagistrasse 10, 8952 Schlieren, Switzerland
[15]ETH Zurich, Institute of Molecular Health Sciences, Otto-Stern-Weg 7, 8093 Zurich, Switzerland
[16]University Hospital Basel, Gynecological Cancer Center, Spitalstrasse 21, 4031 Basel, Switzerland
[17]University Hospital Basel, Institute of Medical Genetics and Pathology, Schönbeinstrasse 40, 4031 Basel, Switzerland
[18]University of Zurich, Faculty of Medicine, Zurich, Switzerland
[19]ETH Zurich, Department of Biology, Wolfgang-Pauli-Strasse 27, 8093 Zurich, Switzerland
[20]These authors contributed equally
[21]These authors contributed equally
*Correspondence: gunnar.raetsch@inf.ethz.ch (G.R.), mitchell.levesque@usz.ch (M.P.L.)
https://doi.org/10.1016/j.ccell.2021.01.004

The application and integration of molecular profiling technologies create novel opportunities for personalized medicine. Here, we introduce the Tumor Profiler Study, an observational trial combining a prospective diagnostic approach to assess the relevance of in-depth tumor profiling to support clinical decision-making with an exploratory approach to improve the biological understanding of the disease.

Introduction

In recent years, the advent of next-generation sequencing (NGS) has allowed cancer centers worldwide to offer personalized treatments, particularly to cancer patients who have no approved treatment options. In this precision oncology approach, off-label treatments are suggested according to the genetic profile of a tumor and are agnostic to the tissue of origin. However, only about one-third of patients show a significant clinical response (Rodon et al., 2019). This calls for approaches to decipher how alterations beyond genetic and epigenetic ones—tumor microenvironment, cellular heterogeneity, and cell-cell interactions—eventually shape tumor growth, vulnerability, and treatment response. The limitations of assessing genetic markers alone have become evident in a basket trial treating *BRAF* V600E-positive malignancies with the BRAF inhibitor Vemurafenib. While the *BRAF* mutation predicts inhibitor efficacy in melanoma, no response was observed in colorectal cancer (Hyman et al., 2015), likely due to feedback activation of the EGFR pathway present in colorectal cancer but absent in melanoma (Prahallad et al., 2012).

Technological progress allows for a comprehensive analysis of the molecular profile and functional responses of tumor cells as well as the composition, spatial organization, and interactions of cells that constitute tumor tissues. These developments have spawned several large-scale initiatives to improve human health (HuBMAP Consortium, 2019; Rajewsky et al., 2020; Rozenblatt-Rosen et al., 2020). However, no existing effort assesses whether cutting-edge technologies can contribute to clinical decision-making in oncology. Here, we introduce the Tumor Profiler (TuPro) study, which

Please cite this article in press as: Irmisch et al., The Tumor Profiler Study: integrated, multi-omic, functional tumor profiling for clinical decision support, Cancer Cell (2021), https://doi.org/10.1016/j.ccell.2021.01.004

we design to deliver an integrated treatment recommendation based on a tumor's high-resolution molecular profile and its *ex vivo* drug response to the tumor board within a clinically relevant turnaround time. This approach has the potential to alter current diagnostics and paves the way for the translation of comprehensive molecular profiling into clinical decision-making.

Study setup

TuPro is an approved, observational clinical study (BASEC: 2018-02050, 2018-02052, 2019-01326) in which we prospectively profile patient tumor samples and assess whether combined multi-omics and functional readouts can provide evidence to support clinical decision-making beyond available and emerging diagnostic technologies such as digital pathology and targeted NGS (Figure 1A). The technologies included in TuPro are selected based on their ability to provide part of a multi-level depiction of the tumor or its microenvironment as well as their potential to deliver robust and clinically relevant insights in short turnaround times. The technologies are applied to 240 tumor samples collected over 3 years across three cancer indications: metastatic melanoma, metastatic epithelial ovarian cancer, and acute myeloid leukemia (AML). The selection of these indications is based on the potential clinical benefit and availability of sufficient tumor material for simultaneous analysis across all technologies (Supplemental Information, Note 1). In addition to multiple bulk approaches, an average of two million single cells per patient are profiled across six technologies with single-cell readouts. The resulting data are analyzed immediately in the context of a "Fast Diagnostic loop," where their relevance to generate treatment recommendations on a per-patient basis is investigated. An in-depth analysis of the data acquired at the cohort level, including the clinical outcome of each patient collected over a 6-month follow-up period, is performed in the context of an "Exploratory Science loop," where we will take advantage of our multiscale approach to improve the understanding of the disease and discover novel biomarkers.

Profiling technologies

We include two emerging clinical diagnostic approaches, i.e., tests that are not yet standard in cancer diagnostics, and seven exploratory profiling technologies in the TuPro study (Figures 1A and S1). Single-cell genomics approaches (scRNA [Papalexi and Satija, 2018] and scDNA [Kuipers et al., 2020]) generate a high-resolution map of the tumor microenvironment, characterize tumor cell heterogeneity, establish each tumor's evolutionary history, and take advantage of insights into cancer genomics and transcriptomics acquired over the past decades. We perform bulk (DIA-MS) proteotyping (Gillet et al., 2012; Xuan et al., 2020) and single-cell CyTOF (Wagner et al., 2019) protein-based analyses not only to expand on and translate transcriptomic observations but also to assess post-translational modifications affecting proteins involved in signaling pathways. The characterization of the tumor microenvironment is enriched with spatially resolved approaches: digital pathology and imaging mass cytometry (IMC) (Giesen et al., 2014) enable the characterization of cell-cell interactions within the tumor microenvironment by providing quantitative, single-cell, and spatially resolved data. This is of particular value for predicting the success of therapies that depend on direct cell-cell interactions, such as immune checkpoint inhibitors. To understand how the comprehensive molecular profile translates into drug sensitivity or resistance, we include two *ex vivo*, single-cell resolution drug response profiling technologies. Pharmacoscopy (Snijder et al., 2017; Vladimer et al., 2017) focuses on cancer-cell-specific drug effectiveness using cell death as a readout, while 4i (iterative indirect immunofluorescence imaging) Drug Response Profiling (Gut et al., 2018) maps the changes in proliferation or survival signaling pathways upon drug treatment, using a multiplexed readout of cancer-relevant molecular markers. Both assays screen a cancer-type-specific set of approved or promising off-label cancer drugs, alone or in combination. Finally, TuPro includes bulk RNA sequencing and targeted DNA sequencing of the tumor and of blood-derived cell-free DNA cfDNA). These well-established cancer-profiling molecular approaches enable the comparison to existing large-scale cohort studies, to leverage their information for patients included in the TuPro study. The unique combination of TuPro technologies overcomes the limitations of current genetic-centric personalized medicine options by providing complementary biomarker data across multiple biological levels and offering a holistic view of a tumor's biology for each individual patient.

Study workflow

All the technology platforms analyze viable fresh frozen tumor material or blood (cf. DNA) from eligible patients in the Fast Diagnostic loop (Figure 1A). These data are then integrated with the results from the emerging clinical diagnostic approaches, i.e., targeted NGS panel sequencing and digital pathology, and clinical data to produce a molecular research report (MRR) for each patient. This report is used in a pre-tumor board (pre-TB), where a multidisciplinary group of physicians generates treatment recommendations based on three levels of evidence: level A, standard clinical guidelines (ESMO clinical guidelines); level B, level A plus emerging clinical diagnostic approaches; and level C, all previous evidence levels plus data from TuPro exploratory technologies. Recommendations for all three levels are recorded and used to assess the usefulness of TuPro, based on defined metrics (see below). These metrics assess whether TuPro data provide actionable information beyond current diagnostics and also, in the longer term, whether this information is correlated with patient outcome. Recommendations based on level C, along with a synopsis of the pre-TB discussion, are communicated to the tumor board. This interdisciplinary expert panel makes the final decision on the best treatment strategy, given all available information on the individual patient.

Clinical follow-up data, such as cancer treatments, side effects, and response data, are used as part of the clinical evaluation of treatment recommendations. Furthermore, these data will be analyzed in conjunction with the molecular data provided by the TuPro technologies within the Exploratory Science loop (Figure 1A), where hypothesis-generating analyses are carried out throughout the study. With its Fast Diagnostic and Exploratory Science loops, TuPro's hybrid nature allows both to recommend actions for each patient based on the identified relevant features and to carry out research activities throughout the study, increasing

Please cite this article in press as: Irmisch et al., The Tumor Profiler Study: integrated, multi-omic, functional tumor profiling for clinical decision support, Cancer Cell (2021), https://doi.org/10.1016/j.ccell.2021.01.004

CellPress

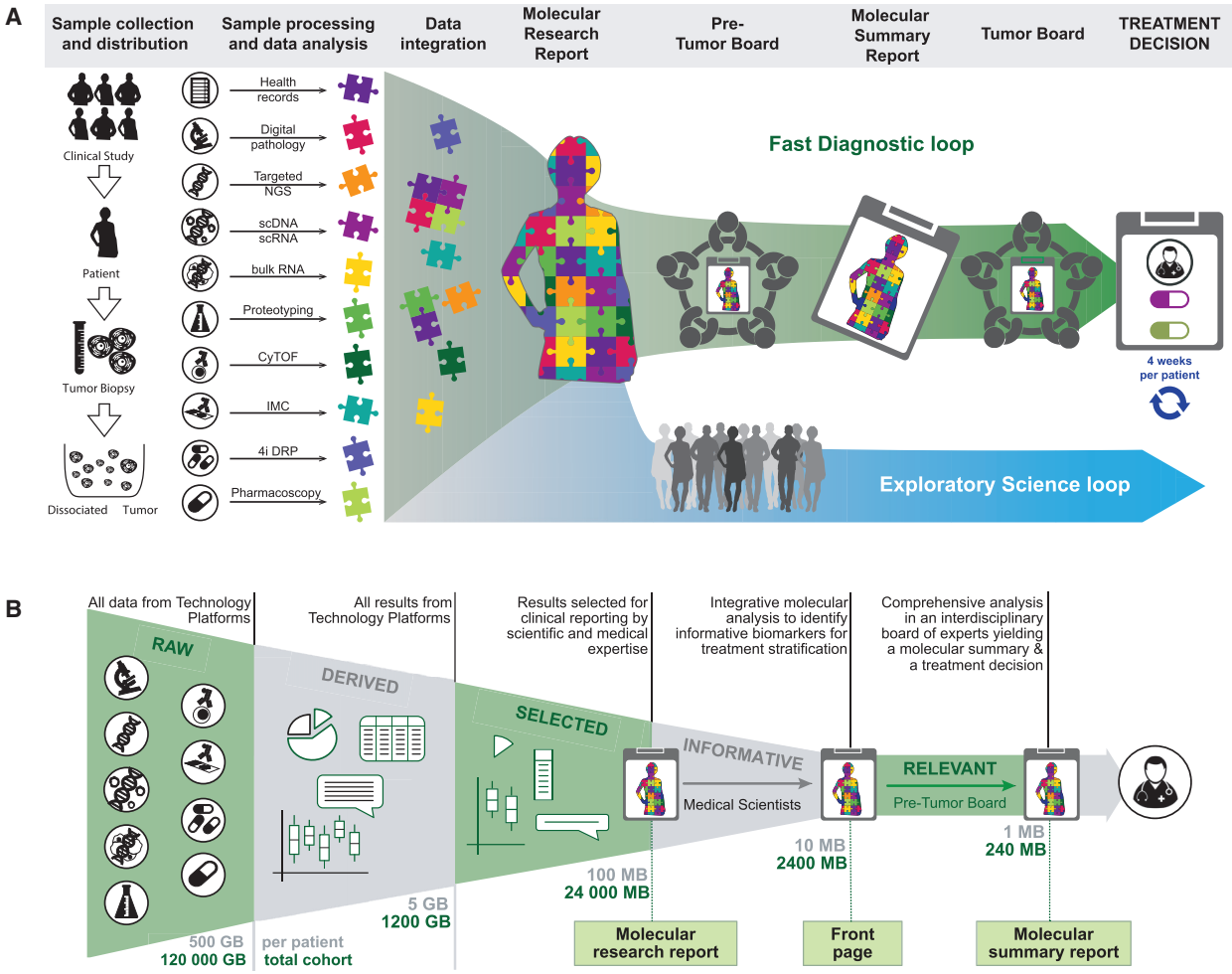

Figure 1. The Tumor Profiler (TuPro) study

(A) Study overview: the study workflow entails patient enrollment, sample collection, analysis by different technology platforms and data integration, creation and discussion of molecular research and summary reports, discussion of treatment options in pre-tumor boards, and the final treatment decision in tumor boards. The study consists of two loops: (1) a Fast Diagnostic loop, which provides integrated information from diagnostic and TuPro exploratory technologies in a 4-week turnaround time from surgery to tumor board; and (2) an Exploratory Science loop, in which cohort analysis is performed during and at the end of the clinical study. 4i DRP, iterative indirect immunofluorescence imaging Drug Response Profiling; CyTOF, mass cytometry; IMC, imaging CyTOF; sc, single-cell.

(B) Schematic representation of the qualitative and quantitative transition from the raw data generated by all TuPro technology platforms to the molecular summary report. The amount of data generated for each patient and overall at each step is indicated below.

the possibilities for new discoveries in cancer biology.

Data analysis and reporting framework for tumor boards

The TuPro study requires a technical and organizational framework for the collection and centralization of molecular and clinical data and for structured reporting to tumor boards. The clinical and molecular data are collected, stored, and analyzed in a customized research data management system. A multidisciplinary team jointly generates the MRR (Figure 1B) based on the collected data and technology-specific analyses. The MRR is made accessible via an interactive web application that provides an overview of potential treatment suggestions along with the specific evidence supporting each option and facilitates discussions between technology experts and clinicians in the pre-TB. A summary of the MRR and the treatment suggestions from the pre-TB is used as a molecular summary report for supporting treatment decisions at the tumor board (Figure 1B). Beyond the clinically driven investigation, the TuPro consortium carries out a deep, discovery-driven analysis of individual and combined technologies to identify new features to improve the understanding of tumor biology and predict treatment responses. In this context, the inclusion of two *ex vivo* drug response assessments at the single-cell level constitutes a notable difference to ongoing efforts and enables the discovery of novel predictive biomarkers. In parallel, we aim at developing new computational models to integrate multimodal single-cell technologies for an unprecedented depth of insight into biological processes, which will constitute relevant resources for the scientific community (Supplemental Information, Note 2).

Advancements in personalized treatment decision support

Treatment decisions based on histopathological analyses and targeted NGS

Please cite this article in press as: Irmisch et al., The Tumor Profiler Study: integrated, multi-omic, functional tumor profiling for clinical decision support, Cancer Cell (2021), https://doi.org/10.1016/j.ccell.2021.01.004

A

		Clinical usefulness parameters	Levels
Throughout TuPro	1	**Overall clinical usefulness** Report changed tumor board decision Usefulness of molecular summary report for decision of treating physicians or Tumor Board panel ESCAT category of tumor board recommendation for genetic markers	[yes/no] [0-5 usefulness scale] [ESCAT category 1-6]
	2	**Technology-specific** Added value for a given treatment recommendation beyond histopathology and targeted NGS	[useful, not useful, not measurable, does not apply]
End of sample analysis phase	3	**Patient-outcome specific** Treatment terminated due to toxicity TTFST ratio (TTFST 2 / TTFST 1) OS (from enrolment until death) Other standard utility metrics typically collected in clinical trials	[yes/no] [greater or lower than 1.3] [months]
End of TuPro	4	**Clinical hypothesis generation**	
	5	**Development of novel clinical biomarkers**	
	6	**Clinical actionability grading based on molecular profiling**	

B

	Routine biomarkers	Emerging biomarkers	Exploratory biomarkers
Definition	• **Defined biomarker** identifying patients likely to benefit from a specific drug (incl. **Companion diagnostics***) • Improved clinical outcome shown in **prospective clinical trials**#	• **Clinical evidence** supporting likely clinical benefit exists • **No** data currently available on **survival endpoints**#	• **Potentially clinically relevant** information (predictive of response, resistance, etc.) • **No** conclusive **clinical data** available#
Common use	• **Standard of care** • **Routine** clinical use	• **Emerging standard** of care • Used by **Molecular Tumor Boards**	• **Preclinical** studies • **Hypothetical** target
Examples	• ***BRCA* mutations** for PARP inhibitors • ***EGFR* amplification** for anti-EGFR antibody • **MSI status** for anti PD1 treatment	• ***PTEN*** loss for PI3K inhibitors • ***AKT1*** activation for AKT inhibitors • **pERK** elevation for MEK inhibitors	• **Pathway activation** scores • Increased **splicing burden** • Others

Figure 2. Clinical applicability of TuPro results

(A) Clinical usefulness is assessed with respect to six different parameters. The recorded levels are listed in the last column. The first two clinical usefulness parameters represent information that is collected and assessed throughout the study. Parameter 3 information is analyzed at the end of the analysis phase, and parameters 4–6 are investigated at the end of the study, once all the information has been integrated. OS, overall survival; TTFST, time to first subsequent treatment; TTFST 1, TTFST on previous treatment (before entering the study); TTFST 2, TTFST on treatment after TuPro.

(B) Molecular biomarker categories. We define three categories of biomarkers based on the level of evidence available on their usefulness ("Definition" row). The "Common use" row defines the current level of usage in the medical diagnostics community, while the last row provides representative examples for each one of the three biomarker categories. The bucket images in the background represent the amount of data available for each category. MSI, microsatellite instability. # as defined in the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT); *FDA: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/vitro-companion-diagnostic-devices.

results are quickly becoming standards in tumor boards. However, our understanding of the complex cellular interactions that comprise the tumor and its microenvironment, as well as its response to targeted or immunotherapies, is still in its infancy. The TuPro consortium is building a state-of-the-art profiling framework that integrates cellular, molecular, spatial, functional, and clinical information from three tumor types and aims to determine the relevance of this in-depth profiling for treatment decisions by a tumor board.

By comparing treatment recommendations based on emerging clinical diagnostic approaches with those that integrate data generated by TuPro, we will assess the potential of TuPro technologies to become part of a new standard for precision medicine. A major goal of TuPro is to clinically evaluate whether the additional molecular profiling informs and improves clinical decision-making due to additional biological insight. First, experts within both the pre-tumor board and the tumor board make an assessment of the clinical usefulness of the data. During these expert evaluations, attending oncologists use utility ratings (Figure 2A, parameters 1 and 2) to score the impact of the data provided by the individual TuPro technologies. At this initial assessment, it would not be possible to relate the information from TuPro

technologies to clinical parameters such as overall survival (OS) or progression-free survival (PFS). At study completion, when outcome data are available, we will evaluate the clinical relevance of TuPro data-based treatment decisions using these clinical outcomes (Figure 2A, parameter 3). The integrative nature of TuPro will also allow for the identification of additional features that could be suggested as novel clinical biomarkers or treatment target candidates, allowing for hypothesis generation and testing within the TuPro framework (Figure 2A, parameters 4–6).

As part of the TuPro study, we identify to which extent features already known to be meaningful for cancer characterization and treatment recommendations are recapitulated in our findings. We consider these as routine biomarkers (Figure 2B, column 1). We expect that integrative analyses of the available TuPro technologies will further provide supportive evidence for emerging biomarkers, defined as novel indicators for clinical management that are not yet fully characterized or established in routine clinical practice (Figure 2B, column 2). We will systematically evaluate known and emerging biomarkers and the corresponding technologies for their inclusion in diagnostic tests. Finally, TuPro will investigate exploratory biomarkers, defined as data for which establishing clinical relevance still requires large studies and complex integration and mining approaches (Figure 2B, column 3). For this purpose, data science and machine-learning algorithms will be leveraged to investigate novel molecular markers associated with drug response, marker expression level as a function of diverse clinical variables, and cell population distribution as a predictor of treatment response, among others. The combination of bulk and single-cell data collected from a multitude of molecular signals offers opportunities for the development of new approaches required for data analysis and integration.

Outlook

The TuPro study is uniquely designed to meet the demands of clinical practice and to produce rich, high-dimensional datasets for in-depth tumor characterization within clinically relevant turnaround times. To achieve swift advances with a direct impact on clinical oncology practice, the corresponding data need to be generated, interpreted, and summarized in fast-paced clinical environments with different ethical, regulatory, and temporal constraints. The TuPro approach could change the way cancer patients are managed by providing novel diagnostic tools and individualized therapies, and it may facilitate the identification of novel prognostic or predictive biomarkers and potential new drug targets.

The cost of deep, multimodal profiling of samples is still high, albeit steadily decreasing. The TuPro infrastructure built in the area of cancer diagnostics today has the potential to become routine in a few years, the same way genome and exome sequencing are now routine tests for the investigation of the molecular basis of genetic disorders. It is of utmost importance to start creating workflows, analytical platforms, and data integration solutions with the aim to leverage the large amount of complex data that will be generated within a clinical framework. We hope that the path pioneered by TuPro will lead the way and complement similar efforts in the pursuit of the successful management of cancer.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.ccell.2021.01.004.

ACKNOWLEDGMENTS

The Tumor Profiler Study is jointly funded by a public-private partnership involving F. Hoffmann-La Roche Ltd., ETH Zurich, University of Zurich, University Hospital Zurich, and University Hospital Basel. We would like to acknowledge the support of the Biobank team from the Department of Dermatology; The Biobank and Tumor Genomic Profiling team of the Department of Pathology and Molecular Pathology at The University Hospital Zurich; and The Biobank and Ovarian Cancer Research at the Department of Biomedicine, University of Basel. Moreover, we acknowledge the support of the Clinical Trials Center (CTC) Zurich and the Clinical Trials Unit (CTU) Basel. We gratefully acknowledge the excellent project management support by the following individuals: Andreas Strauss, Thomas Solbach, Lina Reypens, and Vanessa Mareike Johnen. We thank Natalie de Souza for helping to write the manuscript.

DECLARATION OF INTERESTS

B.S. is scientific co-founder and shareholder of Allcyte GmbH. L.P. and G.G. are listed as inventor on patents related to the 4i technology (WO 2019/207004; WO 2020/008071). G.R., K.-V.L., and S.G.S. are listed on a patent application related to single-cell analyses (European Patent Application No. 20170724.7). H.M. is on advisory boards for Bayer, Astra Zeneca, Janssen, Roche, and Merck. R.D. reports intermittent, project-focused consulting and/or advisory relationships with Novartis, Merck Sharp & Dohme (MSD), Bristol Myers Squibb (BMS), Roche, Amgen, Takeda, Pierre Fabre, Sun Pharma, Sanofi, Catalym, Second Genome, Regeneron, and Alligator outside the submitted work. G.R. is cofounder and on the Scientific Advisory Board of Computomics GmbH. M.P.L. is a co-founder and shareholder of Oncobit AG and receives research funding from Novartis, Roche, and Molecular Partners. The Tumor Profiler study is jointly funded by a public-private partnership involving F. Hoffmann-La Roche Ltd., ETH Zurich, University of Zurich, University Hospital Zurich, and University Hospital Basel.

REFERENCES

Giesen, C., Wang, H.A.O., Schapiro, D., Zivanovic, N., Jacobs, A., Hattendorf, B., Schüffler, P.J., Grolimund, D., Buhmann, J.M., Brandt, S., et al. (2014). Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. Nat. Methods *11*, 417–422.

Gillet, L.C., Navarro, P., Tate, S., Röst, H., Selevsek, N., Reiter, L., Bonner, R., and Aebersold, R. (2012). Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. Mol. Cell. Proteom. *11*, https://doi.org/10.1074/mcp.O111.016717.

Gut, G., Herrmann, M.D., and Pelkmans, L. (2018). Multiplexed protein maps link subcellular organization to cellular states. Science *361*, eaar7042.

HuBMAP Consortium (2019). The human body at cellular resolution: the NIH Human Biomolecular Atlas Program. Nature *574*, 187–192.

Hyman, D.M., Puzanov, I., Subbiah, V., Faris, J.E., Chau, I., Blay, J.-Y., Wolf, J., Raje, N.S., Diamond, E.L., Hollebecque, A., et al. (2015). Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. N. Engl. J. Med. *373*, 726–736.

Kuipers, J., Tuncel, M.A., Ferreira, P., Jahn, K., and Beerenwinkel, N. (2020). Single-cell copy number calling and event history reconstruction. bioRxiv. https://doi.org/10.1101/2020.04.28.065755.

Papalexi, E., and Satija, R. (2018). Single-cell RNA sequencing to explore immune cell heterogeneity. Nat. Rev. Immunol. *18*, 35–45.

Prahallad, A., Sun, C., Huang, S., Di Nicolantonio, F., Salazar, R., Zecchin, D., Beijersbergen, R.L., Bardelli, A., and Bernards, R. (2012). Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature *483*, 100–103.

Rajewsky, N., Almouzni, G., Gorski, S.A., Aerts, S., Amit, I., Bertero, M.G., Bock, C., Bredenoord, A.L., Cavalli, G., Chiocca, S., et al.; LifeTime Community Working Groups (2020). LifeTime and improving European healthcare through cell-based interceptive medicine. Nature *587*, 377–386.

Rodon, J., Soria, J.-C., Berger, R., Miller, W.H., Rubin, E., Kugel, A., Tsimberidou, A., Saintigny, P., Ackerstein, A., Braña, I., et al. (2019). Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. Nat. Med. *25*, 751–758.

Rozenblatt-Rosen, O., Regev, A., Oberdoerffer, P., Nawy, T., Hupalowska, A., Rood, J.E., Ashenberg,

O., Cerami, E., Coffey, R.J., Demir, E., et al.; Human Tumor Atlas Network (2020). The Human Tumor Atlas Network: charting tumor transitions across space and time at single-cell resolution. Cell *181*, 236–249.

Snijder, B., Vladimer, G.I., Krall, N., Miura, K., Schmolke, A.-S., Kornauth, C., Lopez de la Fuente, O., Choi, H.-S., van der Kouwe, E., Gültekin, S., et al. (2017). Image-based ex-vivo drug screening for patients with aggressive haematological malignancies: interim results from a single-arm, open-label, pilot study. Lancet Haematol. *4*, e595–e606.

Vladimer, G.I., Snijder, B., Krall, N., Bigenzahn, J.W., Huber, K.V.M., Lardeau, C.-H., Sanjiv, K., Ringler, A., Berglund, U.W., Sabler, M., et al. (2017). Global survey of the immunomodulatory potential of common drugs. Nat. Chem. Biol. *13*, 681–690.

Wagner, J., Rapsomaniki, M.A., Chevrier, S., Anzeneder, T., Langwieder, C., Dykgers, A., Rees, M., Ramaswamy, A., Muenst, S., Soysal, S.D., et al. (2019). A single-cell atlas of the tumor and immune ecosystem of human breast cancer. Cell *177*, 1330–1345.

Xuan, Y., Bateman, N.W., Gallien, S., Goetze, S., Zhou, Y., Navarro, P., Hu, M., Parikh, N., Hood, B.L., Conrads, K.A., et al. (2020). Standardization and harmonization of distributed multi-center proteotype analysis supporting precision medicine studies. Nat. Commun. *11*, 5248.