

# Functional Precision Medicine Provides Clinical Benefit in Advanced Aggressive Hematologic Cancers and Identifies Exceptional Responders



Christoph Kornauth<sup>1,2</sup>, Tea Pemovska<sup>1,3</sup>, Gregory I. Vladimer<sup>3,4</sup>, Günther Bayer<sup>5</sup>, Michael Bergmann<sup>6</sup>, Sandra Eder<sup>7</sup>, Ruth Eichner<sup>3</sup>, Martin Erl<sup>8</sup>, Harald Esterbauer<sup>9</sup>, Ruth Exner<sup>6</sup>, Verena Felsleitner-Hauer<sup>10</sup>, Maurizio Forte<sup>1</sup>, Alexander Gaiger<sup>1,2</sup>, Klaus Geissler<sup>11</sup>, Hildegard T. Greinix<sup>12</sup>, Wolfgang Gstöttner<sup>13</sup>, Marcus Hacker<sup>14</sup>, Bernd Lorenz Hartmann<sup>15</sup>, Alexander W. Hauswirth<sup>1</sup>, Tim Heinemann<sup>16</sup>, Daniel Heintel<sup>17</sup>, Mir Alireza Hoda<sup>18</sup>, Georg Hopfinger<sup>19</sup>, Ulrich Jaeger<sup>1,2</sup>, Lukas Kazianka<sup>1</sup>, Lukas Kenner<sup>5</sup>, Barbara Kiesewetter<sup>20</sup>, Nikolaus Krall<sup>3,4</sup>, Gerhard Krajnik<sup>21</sup>, Stefan Kubicek<sup>3</sup>, Trang Le<sup>1</sup>, Simone Lubowitzki<sup>1</sup>, Marius E. Mayerhoefer<sup>22,23</sup>, Elisabeth Menschel<sup>24</sup>, Olaf Merkel<sup>5</sup>, Katsuhiko Miura<sup>25</sup>, Leonhard Müllauer<sup>5</sup>, Peter Neumeister<sup>12</sup>, Thomas Noesslinger<sup>24</sup>, Katharina Ocko<sup>26</sup>, Leopold Öhler<sup>27</sup>, Michael Panny<sup>24</sup>, Alexander Pichler<sup>1</sup>, Edit Porpaczy<sup>1</sup>, Gerald W. Prager<sup>2,20</sup>, Markus Raderer<sup>2,20</sup>, Robin Ristl<sup>28</sup>, Reinhard Ruckser<sup>29</sup>, Julius Salamon<sup>30</sup>, Ana-Iris Schiefer<sup>5</sup>, Ann-Sofie Schmolke<sup>1</sup>, Ilse Schwarzinger<sup>9</sup>, Edgar Selzer<sup>31</sup>, Christian Sillaber<sup>1</sup>, Cathrin Skrabs<sup>1</sup>, Wolfgang R. Sperr<sup>1,32</sup>, Ismet Srndic<sup>3</sup>, Renate Thalhammer<sup>9</sup>, Peter Valent<sup>1,32</sup>, Emiel van der Kouwe<sup>1</sup>, Katrina Vanura<sup>1</sup>, Stefan Vogt<sup>10</sup>, Cora Waldstein<sup>31</sup>, Dominik Wolf<sup>33</sup>, Christoph C. Zielinski<sup>34</sup>, Niklas Zojer<sup>17</sup>, Ingrid Simonitsch-Klupp<sup>5</sup>, Giulio Superti-Furga<sup>3,35</sup>, Berend Snijder<sup>16</sup>, and Philipp B. Staber<sup>1,2</sup>

## ABSTRACT

Personalized medicine aims to match the right drug with the right patient by using specific features of the individual patient's tumor. However, current strategies of personalized therapy matching provide treatment opportunities for less than 10% of patients with cancer. A promising method may be drug profiling of patient biopsy specimens with single-cell resolution to directly quantify drug effects. We prospectively tested an image-based single-cell functional precision medicine (scFPM) approach to guide treatments in 143 patients with advanced aggressive hematologic cancers. Fifty-six patients (39%) were treated according to scFPM results. At a median follow-up of 23.9 months, 30 patients (54%) demonstrated a clinical benefit of more than 1.3-fold enhanced progression-free survival compared with their previous therapy. Twelve patients (40% of responders) experienced exceptional responses lasting three times longer than expected for their respective disease. We conclude that therapy matching by scFPM is clinically feasible and effective in advanced aggressive hematologic cancers.

**SIGNIFICANCE:** This is the first precision medicine trial using a functional assay to instruct n-of-one therapies in oncology. It illustrates that for patients lacking standard therapies, high-content assay-based scFPM can have a significant value in clinical therapy guidance based on functional dependencies of each patient's cancer.

See related commentary by Letai, p. 290.

## INTRODUCTION

The overarching goal of precision oncology to match the right treatment with the right patient has prompted clinical trials for patients with refractory cancers to evaluate treatments targeting putative genetic tumor drivers (1–7). In most cancer cases, however, assessment of an individual's underlying genetic disease drivers has not been efficient

at predicting therapy effectiveness, likely because of intra-tumor heterogeneity, dynamic changes, or our incomplete understanding of the genotype-to-phenotype relationship (8–10). Genomic tumor characterization and treatment matching have improved the management of some patients with hematologic malignancies, such as *BRAF* in hairy cell leukemia (11, 12), *IDH1/2* in acute myeloid leukemia

<sup>1</sup>Department of Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria. <sup>2</sup>Comprehensive Cancer Center Vienna, Medical University of Vienna and Vienna General Hospital, Vienna, Austria. <sup>3</sup>CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria. <sup>4</sup>Exscientia GmbH, Vienna, Austria. <sup>5</sup>Department of Pathology, Medical University of Vienna, Vienna, Austria. <sup>6</sup>Department of Surgery, Medical University of Vienna, Vienna, Austria. <sup>7</sup>Department of Internal Medicine and Hematology/Oncology, Klinikum Klagenfurt, Klagenfurt, Austria. <sup>8</sup>Abteilung für Innere Medizin, Krankenhaus der Barmherzigen Brüder Salzburg, Salzburg, Austria. <sup>9</sup>Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria. <sup>10</sup>Department of Medicine and Oncology, LKH Wiener Neustadt, Wiener Neustadt, Austria. <sup>11</sup>Medical School, Sigmund Freud University, Vienna, Austria. <sup>12</sup>Department of Internal Medicine, Division of Hematology, Medical University of Graz, Graz, Austria. <sup>13</sup>Department of Otorhinolaryngology, Head and Neck Surgery, Medical University of Vienna, Vienna, Austria. <sup>14</sup>Division of Nuclear Medicine, Department of Biomedical Imaging and Image-Guided Therapy, Medical University of Vienna, Vienna, Austria. <sup>15</sup>Landeskrankenhaus Feldkirch, Abteilung für Innere Medizin, Feldkirch, Austria. <sup>16</sup>Department of Biology, Institute of Molecular Systems Biology, ETH Zürich, Zürich, Switzerland. <sup>17</sup>Division of Medicine I, Klinik Ottakring, Vienna, Austria. <sup>18</sup>Department of Thoracic Surgery, Medical University of Vienna, Vienna, Austria. <sup>19</sup>Third Medical Department, Centre for Oncology and Haematology, Klinik Favoriten, Vienna, Austria. <sup>20</sup>Department of Medicine I, Division of Oncology, Medical University of Vienna, Vienna, Austria. <sup>21</sup>Department of Medicine I, Universitätsklinikum St. Pölten, St. Pölten, Austria. <sup>22</sup>Department of Biomedical Imaging and Image-Guided Therapy, Medical University of Vienna, Vienna, Austria. <sup>23</sup>Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, New York. <sup>24</sup>Third Medical Department, Hematology & Oncology, Hanusch Hospital, Vienna, Austria. <sup>25</sup>Division of Hematology and Rheumatology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan. <sup>26</sup>Pharmacy Department, Vienna General

Hospital, Vienna, Austria. <sup>27</sup>Internal Medicine I, Department of Oncology, St. Josef Hospital, Vienna, Austria. <sup>28</sup>Section for Medical Statistics, Center for Medical Statistics, Informatics and Intelligent Systems, Medical University of Vienna, Vienna, Austria. <sup>29</sup>Second Medical Department, Klinik Donaustadt, Vienna, Austria. <sup>30</sup>Department of Medicine, Landeskrankenhaus Waidhofen a.d. Ybbs, Waidhofen-Ybbs, Austria. <sup>31</sup>Department of Radiation Oncology, Medical University of Vienna, Vienna, Austria. <sup>32</sup>Ludwig Boltzmann Institute for Hematology and Oncology, Medical University of Vienna, Vienna, Austria. <sup>33</sup>Department of Internal Medicine V, Department of Hematology and Oncology, Medical University of Innsbruck, Innsbruck, Austria. <sup>34</sup>Vienna Cancer Center, Medical University of Vienna, Vienna, Austria. <sup>35</sup>Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria.

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C. Kornauth, T. Pemovska, and G.I. Vladimer contributed equally to this article.

I. Simonitsch-Klupp, G. Superti-Furga, B. Snijder, and P.B. Staber have equal senior authorship of this article.

Current address for R. Eichner: Department of Medicine III, Technical University of Munich, Munich, Germany.

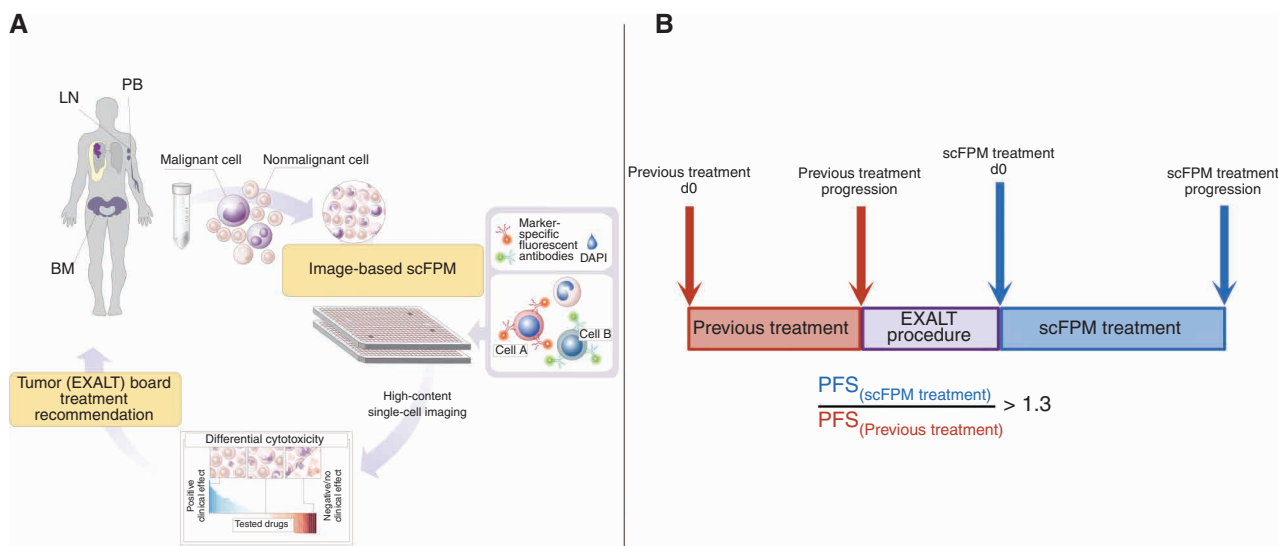
**Corresponding Author:** Philipp B. Staber, Department of Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Währinger Gürtel 18-20, Vienna 1090, Austria. Phone: 43-1-40400 73782; E-mail: philipp.staber@meduniwien.ac.at

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**Figure 1.** EXALT procedure and primary outcome measure. **A**, Viable cells from lymph node (LN), BM, or PB of patients with late-stage hematologic cancer were subjected to image-based scFPM. Target cells are identified by staining with fluorescent antibodies. Reports, automatically generated by the analysis pipeline, are discussed in a dedicated tumor board with patients treated accordingly. **B**, Our primary outcome measure was PFS ratio, defined as  $PFS_{(scFPM\ treatment)}/PFS_{(previous\ treatment)}$ . A ratio of 1.3 is considered individually beneficial. DAPI, 4',6-diamidino-2-phenylindole.

(AML; refs. 13–15), and Philadelphia chromosome in acute lymphoblastic leukemia (16, 17). However, most patients do not currently benefit from therapy selection based on molecular target identification. This is particularly true for patients with relapsed or refractory aggressive disease who still face a dire prognosis (18–20).

Genomically driven drug matching and targeted immunotherapies may be complemented by functional precision medicine strategies, such as high-throughput drug screening approaches, which are agnostic with respect to disease mechanisms and rely on direct measurements of cellular functions (21–24). Single-cell functional precision medicine (scFPM) integrates methods to assess differential cell fates in mixed cell populations derived from patients' real-time biopsy specimens after drug exposure (23). We conducted a prospective trial [Extended Analysis for Leukemia and Lymphoma Treatment (EXALT); NCT03096821] to determine clinical feasibility and the efficacy of scFPM to guide therapy choices for patients with aggressive hematologic cancers who exceeded all standard therapy lines (Fig. 1A). More specifically, we profiled the *ex vivo* efficacy of 139 drugs using high-content microscopy and image analysis on primary patient material to identify resistance-breaking treatments by using a progression-free survival (PFS) ratio of  $\geq 1.3$  as an outcome measure (Fig. 1B; Supplementary Table S1; Supplementary Fig. S1). An interim analysis reported results on the first 17 evaluable patients (23). Here, the final results of the completed EXALT study are presented.

## RESULTS

### Patients

From 2015 to 2019, a total of 193 patients were screened, of whom 143 (74.1%) were eligible, enrolled, and could be tested by scFPM (study population). Seventy-six (53% of

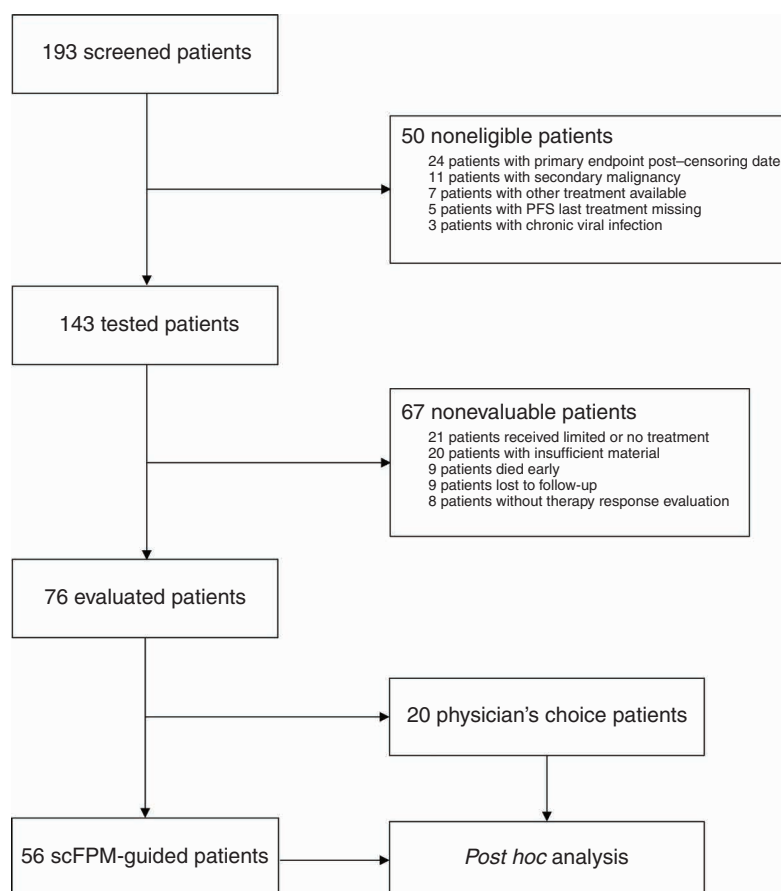
study population; Supplementary Table S2) patients could be evaluated according to study protocol, and 56 (39% of study population, primary analysis set) patients received treatment according to scFPM. Twenty patients received treatment based on physician's choice and thus served as an observational cohort (Fig. 2; Supplementary Table S2).

Fifty-six patients represented the primary analysis data set after having received tumor board–recommended therapy guided by scFPM, and their clinical characteristics are detailed in Table 1. The median age of the patient cohort was 64 years (range, 23–86 years), and the median number of treatment lines before study entry was 3 (1–8). Seventeen patients (30%) had an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or above. The median time was 5 (1–33) days from sampling to scFPM report, with longer times accounting for restaining due to updated immunophenotyping data, and 21 (4–77) days to treatment. The median follow-up was 718 days calculated by the reverse Kaplan–Meier method. Patients had diverse hematologic cancers, both common and rare, such as AML (14/56, 25%), aggressive B-cell non-Hodgkin lymphoma (B-NHL; 26/56, 46%), and T-cell non-Hodgkin lymphoma (T-NHL; 16/56, 28%; Table 1; Supplementary Table S2). Their unifying clinical feature was an aggressive disease, according to the World Health Organization (WHO) classification (25), that lacked standard treatment options.

### Efficacy

Thirty of 56 patients (54%; 95% confidence interval, 40%–67%) from the primary analysis set reached a PFS ratio (PFS on scFPM-guided therapy compared with PFS on prior therapy) of  $\geq 1.3$  with a median PFS ratio of 3.4 [interquartile range (IQR), 2.2–5.7]. This indicates that their individual PFS on scFPM-guided treatment more than tripled compared with their most recent individualized response time. These





**Figure 2.** CONSORT diagram of study patients.

findings led to the rejection of the null hypothesis of less than 15% of patients benefiting from scFPM-guided treatment ( $P < 0.0001$ , one-sided binomial test; Fig. 3A). The PFS on scFPM-guided treatment was significantly increased (HR, 0.58;  $P = 0.0093$ ; Supplementary Fig. S2A). Notably, 13 of 56 patients (23%) were progression-free after 12 months on scFPM-guided therapy compared with 3 of 56 patients (5%) on their previous treatment. The objective response rate (ORR) was 55% for patients treated according to scFPM results, 60% for the lymphoid subgroup, and 41% for patients with myeloid neoplasms (Supplementary Fig. S2B). Eleven patients (~20%) had an ongoing response at the censoring date (Fig. 3A) with a median PFS of 718 days. Moreover, 12 of 56 (21%) scFPM-guided patients experienced exceptional responses, defined as tripled PFS duration compared with expected response duration of the respective disease entity, based on criteria outlined by Wheeler and colleagues (Fig. 3B; ref. 26). Exceptional responders demonstrated better performance status (ECOG  $\leq 1$ ), response [complete response (CR) or partial response (PR)] to prior therapy, and an overrepresentation of a T-NHL diagnosis (7/12; Table 2). For the entire primary analysis cohort, the median PFS ratio was 1.47 (IQR, 0.5–3.51). Eight of 56 (14%) patients received an allogeneous hematopoietic stem cell transplantation (HSCT) or donor lymphocyte infusion (DLI) as a consolidation after reaching

CR on scFPM-guided treatment. This did not translate into a PFS benefit compared with patients in CR who did not receive consolidation with HSCT or DLI (Supplementary Fig. S2C).

Pretreatment performance status influenced benefit from scFPM-guided treatment, with a PFS ratio of  $\geq 1.3$  being reached by 62% of patients with ECOG  $\leq 1$  and by 35% of patients with ECOG  $> 1$  (Fig. 3C; Supplementary Fig. S2D). Median PFS was 207 days for patients with ECOG  $\leq 1$  compared with 29 days for patients with ECOG  $> 1$  (Fig. 3D). Patients who had an objective response (OR) consisting of CR or PR to their previous treatment had a longer PFS on scFPM-guided treatment (Fig. 3E and F). Furthermore, patients with T-NHL had an increased median PFS (235 days) on scFPM-optimized treatment in comparison to patients with B-NHL (60 days) and showed exceptional response in 44% of cases (Supplementary Fig. S2E and S2F; Supplementary Table S2). Somatic *TP53* status was not included in the study protocol but was available for 28 patients. Patients whose cancer harbored a *TP53* variant experienced significantly shorter PFS than those without *TP53* variants (Supplementary Fig. S2G and S2H). The proportion of patients achieving a PFS ratio  $\geq 1.3$  was not significantly influenced by the ECOG performance status, OR to last treatment, lymphoid subgroup, or *TP53* variant status (Supplementary Fig. S3A–S3D).



**Table 1. Characteristics of scFPM-guided patients**

Characteristics	Count	%
All	56	100
Sex		
Male	35	63
Female	21	37
Age, median (range), y	64 (23–86)	
Disease group		
Lymphoma	38	68
Leukemia	18	32
B-NHL	22	39
AML	14	25
T-NHL	15	27
ALL/LBL	5	9
Number of previous treatments, median (range)	3 (1–8)	
Response—last treatment		
CR	15	27
PR	12	21
SD	6	11
PD	23	41
Sampling—treatment in days (range)	21 (4–77)	
ECOG at treatment start		
ECOG 0	17	30
ECOG 1	22	39
ECOG 2	11	12
ECOG 3	6	11
Sample blast fraction		
High (>50%)	34	61
Medium (10%–50%)	13	23
Low (<10%)	8	14
NA	1	2

Abbreviation: ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; NA, information not available; PD, progressive disease; SD, stable disease.

Age ( $\leq 60$  vs.  $> 60$  years), sex, lineage (myeloid vs. lymphoid), number of previous treatment lines ( $\leq 2$  vs.  $> 2$ ), disease subgroup (leukemia vs. lymphoma), and time from sampling to treatment start did not have an impact on PFS duration on scFPM-guided treatment (Supplementary Fig. S4A–S4H). We included a physician's choice cohort of 20 non-scFPM-treated patients who underwent scFPM analysis but, in consultation with their treating physician, decided on alternative treatment. Here, we observed that the PFS prolongation significantly improved in scFPM-treated patients but not in physician's choice patients (Fig. 3G; Supplementary Table S2). Patients treated based on scFPM

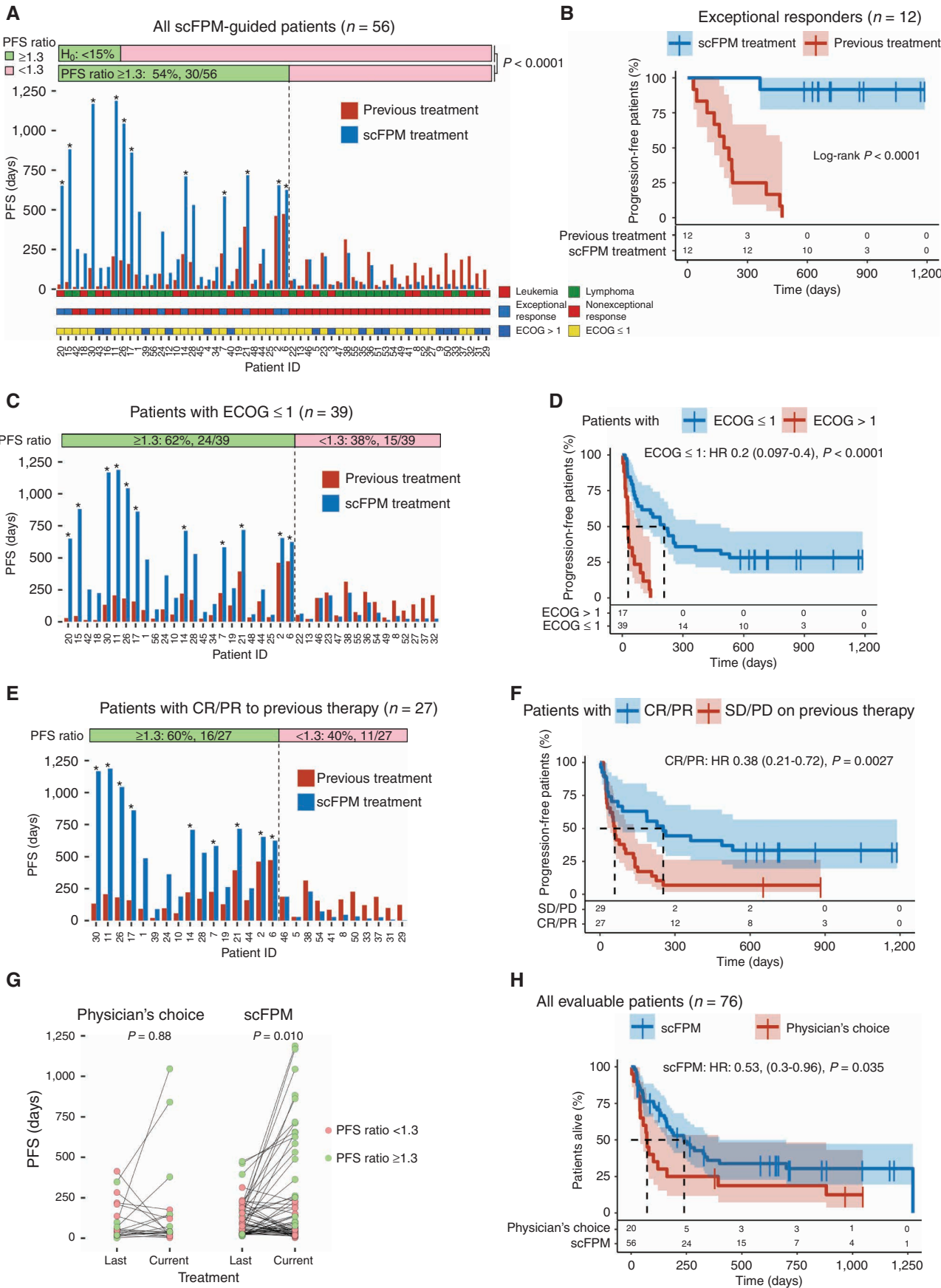
had a significant overall survival benefit compared with the observational cohort (Fig. 3H,  $P = 0.035$ ). Although this study was designed without a controlled comparator arm, the physician's choice cohort was comparable with regard to age, ECOG at treatment start, sample blast fraction, number of previous treatments, and response to previous treatment (Supplementary Fig. S5A–S5E).

### Post Hoc Analysis

To investigate how well the actual received treatment matched the scFPM results by assessment of a matching score, we reanalyzed scFPM image data in a *post hoc* analysis. Updated image analysis pipelines and quality control criteria were used, resulting in the *post hoc* exclusion of 10 patients, with an analysis set of 66 patients consisting of 49 patients from the primary analysis cohort and 17 patients from the physician's choice set. The scFPM results were integrated across evaluated markers and given drugs per patient, leading to an integrated scFPM that was calculated in an automated fashion, blinded to patient outcome. Treatments with positive ( $> 0$ ) integrated scores, denoting overall *post hoc* support for the patient treatment, were considered as matching according to the individual drug-profiling report. Fifty-two patients (78%) obtained such positive scores and were therefore considered as having received scFPM-matched treatment (Supplementary Fig. S6A). Twenty-six of these 52 matched patients (50%) demonstrated an OR to treatment received, and 30 patients had a PFS improvement on scFPM-matched treatment (Supplementary Fig. S6B). Patients receiving matched treatment in the *post hoc* analysis exhibited an increase of PFS. After 12 months, 13 of 52 patients (28%) on matched treatment were progression-free compared with only 4 of 52 patients (8%) on previous treatment (Fig. 4A). Patients receiving non-scFPM-matched treatments did not demonstrate an improved PFS compared with their previous treatment (Fig. 4B). A positively scFPM-matched therapy resulted in a mean PFS of 276 days compared with 121 days on their previous treatment ( $P = 0.0039$ ), whereas non-matched therapy led to a mean PFS of 96 days with a mean previous PFS of 121 days ( $P = 0.51$ ; Fig. 4C).

Influencing factors for PFS were comparable for *post hoc* scFPM-matched treatment and scFPM-guided treatment: ECOG  $\leq 1$ , OR to previous treatment, and lymphoid subtype positively influenced PFS on scFPM-matched treatment. In addition, matched patients with lymphomatous disease had a longer PFS than patients with leukemic disease (Fig. 4D and E; Supplementary Fig. S6C–S6F). We also observed that the relative cancer cell fraction in the sample influenced PFS on scFPM-matched treatment. In particular, patients with

**Figure 3.** scFPM-guided treatment enhances PFS ratio in patients with advanced hematologic cancers and provides a survival benefit. **A**, Bar plot showing the PFS for all included, scFPM-guided patients: blue bars denote PFS in days for scFPM-guided treatment, red bars indicate last previous treatment, and asterisks denote ongoing response for scFPM treatment at the censoring date. PFS ratio is the following ratio:  $\text{PFS}_{\text{scFPM treatment}} / \text{PFS}_{\text{previous treatment}}$ . Patient characteristics are color coded and stratified (leukemia vs. lymphoma, exceptional response vs. nonexceptional response, ECOG  $> 1$  vs. ECOG  $\leq 1$ ). **B**, Kaplan–Meier plot comparing PFS on scFPM-guided treatment with previous treatment in exceptional responders ( $n = 12$ ). **C**, Bar plot showing PFS for all patients with an ECOG  $\leq 1$  ( $n = 39$ ). Asterisks denote ongoing response for scFPM treatment at censoring date. **D**, Kaplan–Meier plot comparing PFS on scFPM treatment between patients with ECOG  $\leq 1$  ( $n = 39$ ) versus ECOG  $> 1$  ( $n = 17$ ). **E**, Bar plot showing PFS for all patients with OR on previous treatment. Asterisks denote ongoing response for scFPM treatment at censoring date. **F**, Kaplan–Meier plot comparing PFS on scFPM treatment stratified according to OR on last treatment (CR/PR:  $n = 27$ , SD/PD:  $n = 29$ ). **G**, Scatter plot comparing PFS on last treatment to current treatment, for scFPM-guided versus physician's choice patients (paired Wilcoxon test). **H**, Kaplan–Meier plot comparing overall survival stratified according to scFPM-guided patients ( $n = 56$ ) versus physician's choice patients ( $n = 20$ ).



**Table 2. Characteristics of exceptional responders**

Characteristics	Count	% of all
Exceptional responders	12	21
Sex		
Male	6	50
Female	6	50
Age, median (range), y	60 (29–86)	
Disease group		
Lymphoma	9	75
Leukemia	3	25
B-NHL	2	17
AML	3	25
T-NHL	7	58
ALL/LBL	0	0
Number of previous treatments, median (range)	2 (2–9)	
Response—last treatment		
CR	7	58
PR	3	25
SD	1	8
PD	1	8
Sampling—treatment in days (range)	28 (4–56)	
ECOG at treatment start		
ECOG 0	8	67
ECOG 1	4	33
ECOG 2	0	0
ECOG 3	0	0
Sample blast fraction		
High (>50%)	4	33
Medium (10%–50%)	6	50
Low (<10%)	1	8
NA	1	8
Sample type		
Bone marrow	2	17
Lymph node	6	50
Peripheral blood	2	17
Skin	1	8
Spleen	1	8

medium cancer cell percentages exhibited longer PFS on scFPM-matched treatment in comparison to patients with either low or high cancer cell percentages (Fig. 4F).

## DISCUSSION

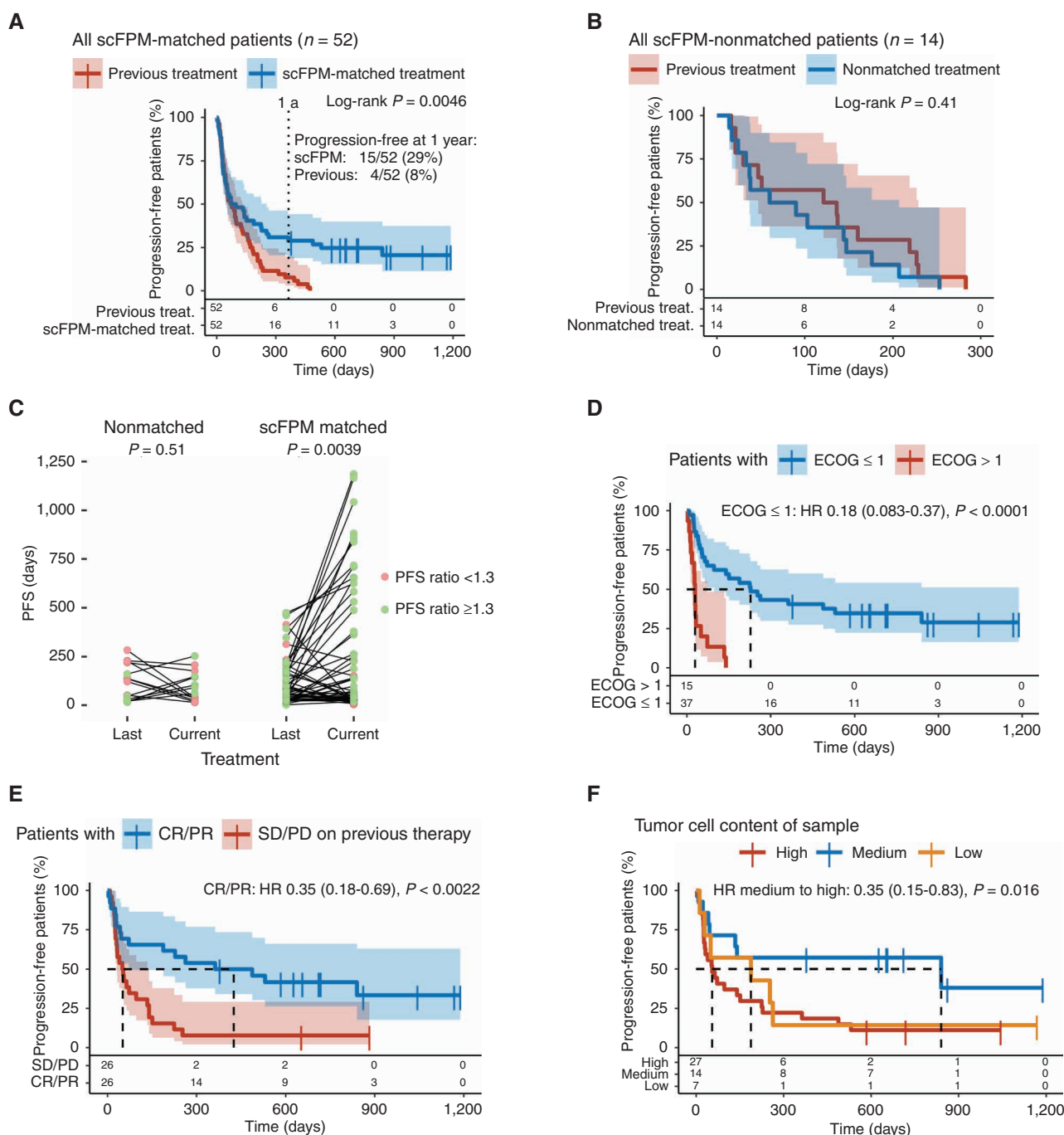
The EXALT trial aimed to offer individualized treatment for patients with aggressive hematologic cancers beyond curative options based on real-time *ex vivo* functional evaluation of drug responses. This single-arm open-label study demonstrates clinical feasibility of integrating an image-based scFPM approach into clinical routine. The primary endpoint of the study was clearly reached. In 54% of cases, scFPM-guided treatments led to a PFS prolongation of  $\geq 1.3$ -fold of the patients' previous individual treatment response time as well as to an enhanced overall survival when comparing scFPM-guided patients with a physician's choice cohort. We found that 21% of patients showed disease-specific exceptional responses,

defined by a threefold extension of absolute PFS duration compared with the expected median PFS duration. This definition was introduced in a study by Wheeler and colleagues (26), which investigated the underlying molecular mechanisms of exceptional responders and identified four broad categories accounting for favorable clinical outcomes: DNA damage response, intracellular signaling, immune engagement, and genetic characteristics of a favorable response. In our study, an ECOG score of 0 or 1 and response to previous treatment were the strongest predictors for a benefit of scFPM-guided treatment. Several clinical parameters such as age, sex, disease lineage, and number of prior treatments did not appear to predict response to scFPM-guided treatment.

Precision medicine trials that aim to match targeted therapies to molecular tumor profiles or gene variants have been emerging in recent years (Table 3; refs. 3, 27–31). Thus far, only few studies could indicate an improved outcome for patients receiving a genetically matched treatment (28, 30, 32). Functional precision medicine trials tailoring treatment strategies based on functional data, such as drug profiling, represent a complementary or alternative strategy and rely on the employed technological platform. Appreciation of the full potential of functional screening technologies, such as the image-based scFPM described here, for their capacity to improve clinical outcome is only now beginning (23). The results of the EXALT study presented here show that scFPM can be an effective tool for clinical decision-making and therapy optimization based on the functional characteristics of each patient's tumor.

Several advantages in comparison to classic sequencing-based approaches can be considered. First, scFPM results were available within days for most patients. We could provide reports for 51 of 76 (67.1%) patients within 7 days. In contrast, a major setback uniformly described in other personalized medicine trials is patient deterioration or death during time of analysis (4, 33). Even in a proof-of-principle, nonoptimized setting, median turnover time for scFPM (5 days) surpassed the limit of current optimized protocols such as the BEAT AML, in which it was possible to deliver genomic analysis within 7 days for 95% of patients (30). In well-optimized settings, scFPM or similar functional approaches can offer reports between 36 and 96 hours post-sampling. Second, no indirect inference from genomic data is needed to design treatment strategies. Matching treatment to genetic mutations may work for only a fraction of cancers in which strong, uniform driver mutations are clearly disease-initiating and disease-maintaining, such as in Philadelphia chromosome-dependent chronic myeloid leukemia. Most of the time, the molecular makeup is complex and intertwined with epigenetic and metabolic states that make correlation to therapeutic outcome impossible at our current state of knowledge (3). Progressive, personalized medicine trials approach this by more general matching scores incorporating common markers for immuno-oncology (e.g., tumor mutational burden, mismatch repair). This strategy could raise the limited matching numbers but has not yet been validated in a prospective fashion (28). In contrast, scFPM provides more accessible readouts, which also take nononcogenic vulnerabilities into account (34). Third, functional drug testing may offer treatment options for a higher number of patients,





**Figure 4.** Post hoc analysis. **A**, Kaplan-Meier plot comparing scFPM-matched treatment with previous treatment. Dotted line denotes 1-year follow-up. **B**, Kaplan-Meier plot comparing non-scFPM-matched treatment with previous treatment. **C**, Paired scatter plot comparing nonmatched versus matched patients with regard to PFS ratio. Paired Wilcoxon test comparing PFS of previous treatment versus scFPM-matched/nonmatched treatment [ $H_0$ : rank  $PFS_{\text{previous}} = \text{rank } PFS_{\text{current}}$ ]. **D**, Kaplan-Meier plot of scFPM-matched treatment stratified according to response to previous treatment. **E**, Kaplan-Meier plot of scFPM-matched treatment stratified according to response to previous treatment. **F**, Kaplan-Meier plots comparing PFS for scFPM-matched patients stratified according to tumor cell content in the sample (high  $\geq 50\%$ , medium  $> 10\%$ , low  $\geq 10\%$ ).

given that clinically validated, approved therapies matched to mutations are available for less than 10% of patients (32, 35, 36), whereas in personalized medicine trials, matched treatment can be allocated to between 4% and 77% (median 24%), dependent on the stringency of matching criteria (Table 3, column 5). Fourth, scFPM-guided therapy can serve as an

effective strategy for rapid remission induction to bridge to stem cell transplantation, which still remains a valid and potentially curative treatment for many hematologic cancers. Fifth, the scFPM platform can systematically identify drug-repurposing opportunities with clinical relevance. In addition, although not done in this work, scFPM can be used for

**Table 3. Summary of precision medicine trials with comparator (randomized, PFS ratio, or observational control)**

Patients screened, <i>n</i>	Patients tested, <i>n</i> (%)	Patients with target availability, <i>n</i> (%)	Patients assigned to treatment, <i>n</i> (%)	Patients treated, <i>n</i> (%)	Patients evaluable for response, <i>n</i> (%)	Response rate	Biomarker type	Comparator	Study
1,893	1,640 (87)	341/827 (41) MALDI-TOF; 583/792 (74) TSACP; 14/21 (67) ASCP	245/1,640 (15)	84/1,640 (5)	ORR, 84/84 (100)	ORR per tested, 16/1,640 (<1) ORR per treatment assigned, 16/245 (6.5) ORR per treated and evaluable, 16/84 (19)	Genetic; IHC; MALDI-TOF MS	Patients treated on genotype-matched trials compared with genotype-unmatched trials	IMPACT/COMPACT (2)
741	520 (70) sequenced; 638 (86) IHC 522 (70) gene copy number analysis 496 (67) complete profile	293/741 (40)	195/741 (26)	99/741 (13.4)	ORR, 98/99 (99)	ORR per tested, 4/293 (1.4) ORR per treatment assigned, 4/195 (2.1) ORR per treated, 4/99 (4) ORR per evaluable, 4/98 (4.1)	Genetic; IHC	Patients randomized to molecularly targeted agent vs. treatment at physician's choice	SHIVA (3)
500	339 (68)	317/339 (93.5)	188/339 (55)	122/339 (36); direct match, 45/339 (13.3)	ORR, 118/122 (97)	ORR per tested, 23/339 (7) ORR per treatment assigned, 23/188 (12) ORR per treated, 23/122 (19) ORR per evaluable, 23/118 (19.5) TTF2/TTF1, NS	Genetic	Matched vs. nonmatched patients; comparison of TTF after enrollment to prior TTF	Genomic Profiling Assay in Phase I (4)
1,542	1,276 (83)	534/1,542 (35)	379/1,276 (30)	143/1,276 (11.2)	ORR, 134/143 (94)	ORR per tested, 16/1,276 (1.3) ORR per treatment assigned, 16/379 (4.2) ORR per treated, 16/143 (11.2) ORR per evaluable, 16/134 (12)	Genetic	Those who had received targeted therapy and those for whom no targeted therapy was available; previously published cohort	Tsimberidou et al. (6)

1,035	843 (81)	411/1,035 (40)	199/843 (24)	199/843 (24)	PFS ratio, 193/199 (97); ORR, 194/199 (97.5)	PFS ratio $\geq 1.3$ per evaluable, 63/193 (33) ORR per enrolled, 22/843 (3) ORR per treated and evaluable, 22/194 (11)	Genetic	Intraindividual PFS ratio (PFS2/PFS1 $>1.3$ )	MOSCATO 01 (7)
106	86 (81)	84/86 (98)	68/86 (79)	66/86 (77)	PFS ratio and ORR, 66/66 (100)	PFS ratio $\geq 1.3$ per evaluable, 18/66 (27) ORR per enrolled, 6/86 (7) ORR per treated and evaluable, 6/66 (9)	Genetic; IHC/ FISH	Intraindividual PFS ratio (PFS2/PFS1 $>1.3$ )	Von Hoff et al. (27)
149	149 (100)	73/149 (49)	83/149 (56)	73/149 (49)	PFS ratio, 53/73 (73); ORR, 69/73 (95)	PFS ratio $\geq 1.3$ per evaluable, 24/53 (45) ORR per tested, 17/149 (11) ORR per treated, 17/73 (23) ORR per evaluable, 17/69 (25)	Genetic; IHC	Intraindividual PFS ratio (PFS2/PFS1 $>1.3$ )	I-PREDICT (28)
303	253 (84)	158/253 (63)	158/253 (62.4)	124/253 (49)	PFS ratio and ORR, 107/124 (86)	PFS ratio $>1.5$ per evaluable, 24/107 (22.4); ORR per tested, 15/253 (6) ORR per assigned to treatment, 15/158 (9.5); ORR per treated, 15/124 (12) ORR per evaluable, 15/107 (14)	Genetic	Intraindividual PFS ratio (PFS2/PFS1 $>1.5$ )	WINTHER (29)
487	487 (100)	317/487 (65)	395/487 (81)	224/487 (46)	OS, 395/487 (81)	30-day mortality, 8/395 (2) Median OS, 12.8 months OS estimate at 12 months, 54.7% No ORR reported	Genetic; IHC	Standard of care, palliative and investigational therapy vs. BEAT-AML substudy	BEAT-AML (30)

(continued)



Table 3. Summary of precision medicine trials with comparator (randomized, PFS ratio, or observational control) (Continued)

Patients screened, n	Patients tested, n (%)	Patients with target availability, n (%)	Patients assigned to treatment, n (%)	Patients treated, n (%)	Patients evaluable for response, n (%)	Response rate	Biomarker type	Comparator	Study
1,856	1,223 (66)	282/1,223 (23)	677/1,223 (55.4)	46/1,223 (4)	05, 677/1,223 (55.4)	Median OS matched therapy group vs. nonmatched group (2.58 vs. 1.51 years) Median PFS, 10.93 vs. 4.53 months	Genetic	Patients treated on matched therapy vs. treated with nonmatched therapy	Know Your Tumor Registry (32)
2,340	1,484 (63)	1,153/1,484 (78)	1,138/1,484 (77)	362/1,484 (24.4)	PFS ratio, 300 (13); best response comparison, 181 (8)	No ORR reported PFS ratio >1.3, 107/300 (36) ORR, 43/181 (24) DCR, 100/181 (55)	Genetic	Intraindividual PFS ratio (PFS2/PFS1 >1.3)	MASTER (38)
193	143 (74)	143/143 (100)	76/143 (53)	56/143 (39)	PFS ratio and ORR, 56/143 (39)	PFS ratio ≥1.3, 30/56 (54) ORR per tested, 31/143 (22) ORR per treated and evaluable, 31/56 (55.4)	Functional	Intraindividual PFS ratio (PFS2/PFS1 >1.3)	EXALT-1 (this study)

NOTE: Patients screened: number of patients who enrolled or signed the consent form. Patients tested: number of patients on whom the test was performed. Patients with target availability: number of patients in whom an actionable target was detected. Patients assigned to treatment: number of patients with a treatment recommendation. Patients treated matched: number of patients treated according to treatment recommendation. Patients evaluable for response: number of patients evaluable for outcome. Response rate: reporting on outcome per tested, treatment assigned, treated, and/or evaluable. Biomarker type: type of biomarker used in study for treatment matching. Comparator: what the outcome was compared with (control arm, intraindividual benefit, or observational cohort). Study: reference to original study.

Abbreviations: ASCP, AmpliSeq Cancer Panel; DCR, disease control rate; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight mass spectrometer; MS, mass spectrometry; NS, not significant; OS, overall survival; TSACP, TruSeq Amplicon Cancer Panel; TTF, time to treatment failure.

combinatorial drug testing. As most effective cancer therapies rely on a combination of agents, means to easily test combinatorial efficacy of drugs are urgently needed.

Despite the advantages, there are limitations to this approach. For instance, scFPM is based on the collection of viable cells, the procurement of which requires an intimate interplay between different hospital departments, such as surgery, pathology, and laboratory. However, as the concept of temporal tumor evolution is more and more recognized and thus real-time biopsy becomes common for personalized approaches, this hurdle can be expected to vanish gradually. Furthermore, we acknowledge the diversity of our patients with regard to disease histology, as a heterogeneous patient population was analyzed with many different neoplasms. To circumvent this, we used a PFS ratio as the primary end-point, based on considerations of Von Hoff and colleagues (27) and Bailey and colleagues (37) and accepted by regulatory bodies ([https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-evaluation-anticancer-medicinal-products-man-revision-6\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-evaluation-anticancer-medicinal-products-man-revision-6_en.pdf)). The use of each patient as his or her own control allows us to evaluate the individual benefit, and it became common in precision medicine trials, such as the WINTHER and MASTER trials including heterogeneous cancer entities (29, 38). Another limitation of the study is that most patients were heavily pretreated, which may have limited their ability to respond. Given the trial's nonrandomized design, we cannot exclude that prognostic or other aspects may have confounded the findings. We did not directly assess the activity of drug combinations *ex vivo* but could still successfully design drug combination treatment strategies that had a positive effect on the PFS *in vivo*. We focused on cell death as our assay readout and thus might have missed compounds that exhibit anticancer effects by other mechanisms. Moreover, the entire surface of each well in the plate was not imaged, and we tested a limited concentration range, but initial optimization experiments indicated that the obtained data were representative of the entire well. The wider applicability and scalability of the approach are currently being evaluated in additional studies. We envision a future deployment of the technology to centralized laboratories. Last, multiple-testing adjustments were not performed, and thus these data would need to be confirmed by future studies.

The EXALT trial shows that functional testing can be integrated into clinical workflows and provide individual benefit for patients with late-stage hematologic cancer. These platforms appear especially suited for hematologic malignancies, as primary patient material is more readily accessible as intact viable cells. Thus, it makes it possible to introduce a class of assays traditionally related to drug discovery and translational research to personalized medicine. Complementation of classic histology and molecular -omics data with functional assays such as scFPM could lead to a comprehensive way of cancer diagnosis and ultimately to improved patient care and outcome in an increasingly predictive manner. In particular, matching phenotypic characterization to molecular profiles may lead to a constantly improving molecular-mechanistic understanding. Perhaps an integrative approach combining molecular and functional profiles may represent an ideal precision medicine

approach, and this remains to be explored in future studies and trials.

An important aspect for success of these approaches is a well-functioning multidisciplinary tumor board, which was implemented in this study and served as a key instrument to optimize individualized treatment strategies. Data integration of a *post hoc* analysis could confirm the matching of the guiding scFPM test results with the treatment actually received (Fig. 4A and B). This work is the basis for the recently initiated prospective randomized trial comparing scFPM with comprehensive genomic profiling and physicians' choice (EXALT-2, NCT04470947).

## METHODS

### Patients

In this open-label, one-arm study, we enrolled patients with confirmed aggressive hematologic cancers according to the WHO classification who had received at least two lines of treatment or had no standard therapy options. All patients provided written informed consent. Real-time biopsy specimens were obtained from every enrolled patient.

### Study Oversight and Conduct

The study was approved by the independent ethics committee at the Medical University of Vienna (institutional review board votes: EK 1830/2015, 2008/2015, 1895/2015) and was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. The study was designed by one author (P.B. Staber) and the sponsor (Medical University of Vienna, Vienna, Austria). The first and last authors wrote all manuscript drafts. All authors vouch for the completeness and accuracy of the data and the adherence of the study to the protocol.

### Image-Based scFPM

Cancer cell-containing tissue was procured by biopsy, bone marrow aspirate, or peripheral blood draws (Fig. 1A). Single-cell suspensions of biopsy material containing tumor cells were suspended in RPMI containing 10% FBS and 1% penicillin-streptomycin, and 20,000 cells per well were plated on 384-well CellCarrier ultra-imaging plates (PerkinElmer) containing 136 to 139 drugs prespotted with an Echo (Labcyte; Supplementary Fig. S1; Supplementary Table S1) and incubated overnight (18–24 hours) at 37°C and 5% CO<sub>2</sub>. The drugs were tested in two different concentrations (1,000 nmol/L and 10,000 nmol/L) in duplicate or triplicate, respectively. After the incubation period, the cells were fixed with 0.5% formaldehyde and 1:1,000 Triton X in PBS, stained with 4',6-diamidino-2-phenylindole (Thermo Fisher Scientific) for nuclear identification, and stained with antibodies to identify if the cell was malignant or healthy. The antibodies used to detect the target cancer cell population were based on established antigens for a given indication, as well as on pathology or laboratory medicine reports for each individual patient. Immunofluorescence staining, imaging by automated microscopy (Opera Phenix; Perkin Elmer), image analysis (CellProfiler; Broad Institute), data analysis (MATLAB), and quality control were done as described previously (23, 39). In short, each cell in the images was identified using single-cell image analysis and subjugated to machine learning-based single-cell quality control. Based on the marker expression levels, cells were scored as being either cancer (marker positive) or healthy. The fraction of cancer cells upon *ex vivo* drug treatment was subsequently compared with the cancer fraction in DMSO controls [leading to a relative cancer fraction (RCF)]. The RCFs were averaged across concentrations and replicates per drug

and subsequently transformed to 1-RCF, such that positive scores denote on-target reduction of cancer cells induced by the *ex vivo* drug treatment.

### Treatment Allocation

The results of the scFPM platform were presented at a formal multidisciplinary tumor board consisting of hematologists, pathologists, specialists from laboratory medicine, biologists, and pharmacists. The board then issued written treatment recommendations directly to the case manager and the treating physician (Fig. 1A). For the observational cohort, patients were treated according to physician's choice based on individual diagnosis and biomarkers (genetic, IHC, and FACS). Treatment was given according to European Union and Austrian legislation as an individual healing attempt within the named patient program and under the responsibility and supervision of the treating physician.

### Assessments and Endpoints

Individual patient benefit was measured by the PFS ratio, defined as PFS achieved on scFPM-guided therapy to PFS observed on previous therapy  $\left( = \frac{\text{PFS (scFPM treatment)}}{\text{PFS (previous treatment)}} \right)$ . A PFS ratio of  $\geq 1.3$

was considered beneficial based on considerations of Bailey and colleagues (37) and Von Hoff and colleagues (27), thereby using each patient as his or her individual control, which is a study endpoint for precision medicine studies as recommended by health agencies such as the European Medicines Agency and others. Our null hypothesis was that less than 15% of patients meet the primary endpoint, a common level of benefit for personalized medicine trials (1, 40). PFS was computed as the time from the first day of treatment to the date of first reported evidence of disease progression or relapse, initiation of new (unplanned) anticancer treatment, or death as a result of any cause. Commonly used and predefined sequential treatments (e.g., 3 + 7 followed by high-dose cytarabine followed by HSCT in AML) were considered as one line, and the PFS durations were summed. Patients not being able to reach the primary endpoint by the censoring date (January 30, 2020) were excluded regardless of response state. For the *post hoc* analysis, patients were assessed in terms of their actual received treatment and its matching level to the scFPM results. The scFPM imaging data from all evaluated patients were reanalyzed with upgraded image analysis pipelines and quality control criteria, which resulted in the exclusion of 10 patients. Therefore, the final *post hoc* set comprised 49 patients from the primary analysis cohort and 17 patients from the physician's choice set. The matching score of the *post hoc* analysis was calculated in an automated fashion, blinded to patient outcome. Sample blast fraction was determined histologically or from flow cytometry data using a three-tiered scale ( $\leq 10\%$ , low;  $\leq 50\%$ , medium;  $> 50\%$ , high).

### Response Evaluation

Response evaluation was based on response evaluation criteria in lymphoma (RECIL) and European LeukemiaNet (ELN) criteria for leukemia (41, 42). As patients with leukemia were not consistently followed up with bone marrow (BM) biopsy or aspirate, changes in peripheral blood (PB) were used equivalently: CR could not be stated with presence of blasts in PB. Analogous to ELN-morphologic leukemia-free state, absence of blasts in the peripheral blood without BM available was considered a hematologic leukemia-free state (HLFS) and classified as an OR. Comparable to morphologic criteria with ELN-PR, reduction of blasts in PB by at least 50% was regarded as PR [hematologic partial remission (HPR) = PR]. The white blood cell count had to be above 1 G/L for HLFS and HPR. ECOG performance status was extracted from patient charts and referral reports where available, or a median score of four independent reviewers blinded

to outcome was used, based on chart notes, nursing reports, and discharge letters.

Exceptional response was defined as tripled PFS duration compared with median PFS for a given diagnosis. For T-NHL, 9 months of PFS was considered exceptional, and for aggressive B-NHL and myeloid disease, 18 months was considered exceptional based on the definition by Wheeler and colleagues (26) and others (18, 43, 44).

Response assessment was reviewed by an internal study committee consisting of three hematologists (P.B. Staber, K. Miura, U. Jaeger), a radiologist (M.E. Mayerhoefer), and a pathologist (C. Kornauth). Somatic mutation *TP53* status was obtained from reports for routine diagnostics produced using certified tests in laboratories of either clinical or surgical pathology.

### Statistical Analysis

The primary endpoint of this study was the percentage of patients reaching a PFS ratio of  $\geq 1.3$  with an  $H_0$  hypothesis of  $< 15\%$  patients meeting the primary endpoint. To test this hypothesis, a one-sided binomial test was applied with an  $\alpha$  of 0.025. The null hypothesis could be rejected when at least 14 of 50 patients showed a PFS of  $\geq 1.3$ . The predefined secondary endpoints were ORR, disease control rate, and PFS, including subgroup analysis (diagnostic group, performance status, age, sex, number of prior therapies, relative blast fraction). The analysis for exceptional responders was not preplanned. A detailed description of the analysis plan can be found in the supplementary materials. All statistical analyses were performed using the R statistical environment (R Core Team; R Foundation for Statistical Computing) and MATLAB (MathWorks). Survival times were compared using the Kaplan-Maier estimator, log-rank tests, and Cox proportional hazard models. Continuous variables were compared with Wilcoxon rank-sum tests. A *P* value of  $< 0.05$  was considered significant.

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## Authors' Contributions

**C. Kornauth:** Resources, data curation, formal analysis, supervision, validation, investigation, visualization, methodology, writing—

original draft, project administration, writing—review and editing. **T. Pemovska:** Data curation, formal analysis, validation, investigation, writing—original draft, project administration, writing—review and editing. **G.I. Vladimer:** Formal analysis, validation, investigation, methodology, writing—original draft. **G. Bayer:** Resources, investigation. **M. Bergmann:** Resources, investigation. **S. Eder:** Resources, investigation. **R. Eichner:** Resources, investigation. **M. Erl:** Resources, investigation. **H. Esterbauer:** Resources, investigation. **R. Exner:** Resources, investigation. **V. Felsleitner-Hauer:** Resources, investigation. **M. Forte:** Resources, investigation. **A. Gaiger:** Resources, investigation. **K. Geissler:** Resources, investigation. **H.T. Greinix:** Resources, investigation. **W. Gstöttner:** Resources, investigation. **M. Hacker:** Resources, investigation. **B.L. Hartmann:** Resources, investigation. **A.W. Hauswirth:** Resources, investigation. **T. Heine-mann:** Resources, investigation. **D. Heintel:** Resources, investigation. **M.A. Hoda:** Resources, investigation. **G. Hopfinger:** Resources, investigation. **U. Jaeger:** Conceptualization, resources, investigation. **L. Kazianka:** Resources, data curation, investigation, project administration. **L. Kenner:** Resources, investigation. **B. Kiesewetter:** Resources, investigation. **N. Krall:** Conceptualization, resources, data curation, software, formal analysis, validation, investigation, methodology, project administration. **G. Krajnik:** Resources, investigation. **S. Kubicek:** Resources, investigation, methodology. **T. Le:** Resources, investigation. **S. Lubowitzki:** Data curation, investigation. **M.E. Mayerhoefer:** Resources, investigation, visualization. **E. Menschel:** Resources, investigation. **O. Merkel:** Resources, investigation. **K. Miura:** Resources, data curation, investigation. **L. Müllauer:** Resources, investigation. **P. Neumeister:** Resources, investigation. **T. Noesslinger:** Resources, validation. **K. Ocko:** Resources, investigation. **L. Öhler:** Resources, investigation. **M. Panny:** Resources, investigation. **A. Pichler:** Resources, investigation, project administration. **E. Porpaczy:** Resources, investigation. **G.W. Prager:** Resources, investigation. **M. Raderer:** Resources, investigation. **R. Ristl:** Conceptualization, data curation, formal analysis, visualization, methodology. **R. Ruckser:** Resources, investigation. **J. Salamon:** Resources, investigation. **A.-I. Schiefer:** Resources, investigation. **A.-S. Schmolke:** Resources, data curation, investigation, project administration. **I. Schwarzinger:** Resources, investigation. **E. Selzer:** Resources, investigation. **C. Sillaber:** Resources, investigation. **C. Skrabs:** Resources, investigation. **W.R. Sperr:** Resources, investigation. **I. Srndic:** Resources, data curation, formal analysis, validation, investigation. **R. Thalhammer:** Resources, investigation. **P. Valent:** Resources, investigation. **E. van der Kouwe:** Resources, data curation, investigation, project administration. **K. Vanura:** Resources, investigation. **S. Vogt:** Resources, investigation. **C. Waldstein:** Resources, investigation. **D. Wolf:** Resources, investigation. **C.C. Zielinski:** Resources, investigation. **N. Zojer:** Resources, investigation. **I. Simonitsch-Klupp:** Resources, data curation, investigation, project administration, writing—review and editing. **G. Superti-Furga:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, writing—original draft, writing—review and editing. **B. Snijder:** Conceptualization, resources, data curation, software, formal analysis, supervision, validation, investigation, visualization, methodology, writing—original draft, project administration, writing—review and editing. **P.B. Staber:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing—original draft, project administration, writing—review and editing.

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