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

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Conflict of interest
GV, NK, GSF, and BS are founders of Allcyte GmbH and shareholders of Exscientia Ltd., and are inventors on patents EP 3 704 484 A1 (GV, NK, GSF) and EP 3 198 276 A1 (BS, GV, GSF) and related patent family members pertaining to this work; the patent families were previously assigned to Allcyte GmbH and now are fully controlled by Exscientia Ltd. through the acquisition of Allcyte GmbH. GV and NK are employees of Exscientia GmbH, formerly Allcyte GmbH, a fully owned subsidiary of Exscientia Ltd. KM reports personal fees from Chugai, Kyowahakko Kirin, outside the submitted work. KO reports personal fees from Novartis, outside the submitted work. UJ reports personal fees from Janssen Cilag, during the conduct of the study; grants and personal fees from Roche, Celgene, Gilead, Novartis, and True North Therapeutics; and personal fees from Amgen, Takeda, AbbVie, Infinity, outside the submitted work. GSF reports grants from ERC Proof of Concept, during the conduct of the study; BS receives research funding from F. Hoffmann-La Roche AG (Tumor Profiler Consortium). PBS receives research funding from F. Hoffmann-La Roche AG and personal fees from Amgen, Takeda, AbbVie, Janssen Cilag, Incyte, Celgene, BMS, Roche, MSD, and Astra-Zeneca, outside the submitted work. All other authors declare no competing interests.
Abstract

Personalized medicine aims to match the right drug with the right patient by utilizing specific features of the individual patients’ tumor. However, current strategies of personalized therapy matching only provide treatment opportunities for less than 10% of cancer patients. A promising method may be drug profiling of patient biopsies 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 (PFS) compared to 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 single-cell functional precision medicine (scFPM) can have a significant value in clinical therapy guidance based on functional dependencies of each patients’ cancer.
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 due to intratumor heterogeneity, dynamic changes, or our incomplete understanding of the genotype to phenotype relationship (8–10). Genomic tumor characterization and treatment matching has improved the management of some patients with hematological malignancies, such as BRAF in hairy cell leukemia (11,12); IDH1/2 in acute myeloid leukemia (AML) (13–15) and Philadelphia chromosome in acute lymphoblastic leukemia (16,17). However, the vast majority does not currently benefit from therapy selection based on molecular target identification. This is particularly true for patients suffering from 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 in 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 biopsies 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 PFS ratio of
≥1.3 as an outcome measure (Fig. 1B, Supplementary Table 1, 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 which 143 (74.1%) were eligible, enrolled, and could be tested by scFPM (study population). Seventy-six (53% of study population; Supplementary Table 2) 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 2).

Fifty-six patients represented the primary analysis dataset after having received tumor-board recommended therapy guided by scFPM, whose clinical characteristics are detailed in Table 1. The median age of the patient cohort was 64 years (range 23-86) and the median number of treatment lines before study entry was 3 (1-8). Seventeen patients (30%) had an ECOG performance status of 2 or above. The median time from sampling to scFPM report was 5 (1-33) days, with longer times accounting for re-staining due to updated immunophenotyping data, and to treatment 21 (4-77) days. The median follow-up was 718 days calculated by the reverse Kaplan-Meier method. Patients suffered from diverse hematological cancers, both common and rare, such as acute myeloid leukemia (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 2). Their unifying clinical feature was an aggressive disease according to the WHO classification (25) lacking standard treatment options.

Efficacy

Thirty out of 56 patients (54%, CI: 40%-67%) from the primary analysis set reached a PFS ratio (PFS on scFPM-guided therapy compared to PFS on prior therapy) of ≥1.3 with a
median PFS ratio of 3.4 (IQR 2.2–5.7). This indicates that their individual PFS on scFPM
guided treatment more than tripled when compared to their most recent individualized
response time. These 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 out of 56 patients (23%) were progression free after 12
months on scFPM-guided therapy compared to 3 out 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%) of scFPM
guided patients experienced exceptional responses, defined as tripled PFS duration compared
to expected response duration of the respective disease entity, based on criteria outlined by
Wheeler et al (Fig. 3B)(26). Exceptional responders demonstrated better performance status
(ECOG≤1), response (CR or 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 allogeneic 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 when
compared to patients in CR that 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 ≥1.3 being reached by 62% of patients with ECOG≤1 and by 35% of patients with
ECOG>1 (Fig. 3C and Supplementary Fig. S2D). Median PFS was 207 days for patients with
ECOG≤1 compared to 29 days for patients with ECOG>1 (Fig. 3D). Patients that had an OR
consisting of CR or PR to their previous treatment had a longer PFS on scFPM-guided treatment (Fig. 3E,F). Furthermore, patients suffering from 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,F, Supplementary Table 2). 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,H). The proportion of patients achieving a PFS-ratio ≥1.3 was not significantly influenced by the ECOG performance status, OR to last treatment, lymphoid subgroup or TP53 variant status (Supplementary Fig. S3A-D).

Age (≤60 versus >60), sex, lineage (myeloid versus lymphoid), number of previous treatment lines (≤2 versus >2), disease subgroup (leukemia versus lymphoma), and time from sampling to treatment start did not have an impact on PFS duration on scFPM-guided treatment (Supplementary Fig. S4A-H). We included a physician’s choice cohort of 20 non-scFPM treated patients, that 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 2). Patients treated based on scFPM had a significant overall survival benefit compared to 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.5A-E).

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, resulting in 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. Treatment(s) 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 out 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 out of 52 patients (28%) on matched treatment were progression free compared to only 4 of 52 patients (8%) on previous treatment (Fig. 4A). Patients receiving non-scFPM-matched treatments did not demonstrate an improved PFS compared to their previous treatment (Fig. 4B). A positively scFPM-matched therapy resulted in a mean PFS of 276 days compared to 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≤1, OR to previous treatment and lymphoid subtype positively influenced PFS on scFPM-matched treatment. Additionally, matched patients suffering from lymphomatous disease had a longer PFS than patients with leukemic disease.
We also observed that the relative cancer cell fraction in the sample influenced PFS on scFPM-matched treatment. In particular, patients with 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 hematological 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 ≥1.3-fold of the patients’ previous individual treatment response time as well as to an enhanced overall survival when comparing scFPM-guided patients to 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 to the expected median PFS duration. This definition was introduced in a study by Wheeler et al. 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 (26). 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) (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 patients’ tumor.

Several advantages in comparison to classical sequencing-based approaches can be considered. First, scFPM results were available within days for the majority of patients. We could provide reports for 51 out 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, non-optimized setting, median turnover time for scFPM (5 days) surpassed the limit of current optimized protocols like the BEAT-AML where 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 only work for a fraction of cancers and treatments, where strong, uniform driver mutations are clearly disease-initiating and disease-maintaining, such as in Philadelphia-chromosome dependent chronic myeloid leukemia (CML). 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).
personalized medicine trials approach this by more general matching scores incorporating common markers for immune-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 non-oncogenic vulnerabilities into account (34). Third, functional drug testing may offer treatment options for a higher number of patients given that clinically-validated approved therapies matched to mutations are available for less than 10% of patients (32,35,36), while in personalized medicine trials matched treatment can be allocated between 4-77% (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 hematological 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 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 primary endpoint, based on considerations of Von Hoff et al. and Bailey et al. and accepted by regulatory bodies (27,37 and
The EXALT trial shows that functional testing can be integrated into clinical workflows and provide individual patient benefit for late-stage hematological cancer patients. These platforms appear especially suited for hematological 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 classical 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
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 an post-hoc analysis could confirm the
matching of the guiding scFPM test results with the actually received treatment (Fig. 4A,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
hematological 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 biopsies 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 (IRB 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 PBS and the sponsor
(Medical University of Vienna). 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 either 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% fetal bovine serum (FBS) and 1% penicillin/streptomycin and 20,000 cells per well were plated on 384-well CellCarrier ultra imaging plates (PerkinElmer) containing 136-139 drugs pre-spotted with an Echo (Labcyte) (Supplementary Fig. 1, Supplementary Table 1) and incubated overnight (18-24h) at 37°C and 5% CO₂. The drugs were tested in two different concentrations (1000nM and 10000nM) in duplicate or triplicate, respectively. After the incubation period, the cells were fixed with 0.5% Formaldehyde and 1:1000 Triton X in phosphate buffered saline (PBS) and stained with 4′,6-Diamidin-2-phenylindol (DAPI, Thermo Fisher Scientific) for nuclear identification, and stained with antibodies to identify if the cell is 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; PerkinElmer), 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 to 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 EU 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 end points

Individual patient benefit was measured by the progression free survival (PFS)-ratio defined as PFS achieved on scFPM-guided therapy to PFS observed on previous therapy ($\frac{\text{PFS} \text{(scFPM treatment)}}{\text{PFS} \text{(previous treatment)}}$). A PFS ratio of $\geq 1.3$ was considered beneficial based on considerations of Bailey et al. and Von Hoff et al. (27,40) thereby using each patient as their individual control, which is a study end-point for precision medicine studies as recommended by health agencies such as the European Medicines Agency (EMA) 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 (1,41). PFS was computed as the time from first day of treatment to either 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 hematopoietic stem-cell transplantation (HSCT) in AML are considered as one line, and the PFS durations were summed up. Patients not being able to reach the primary endpoint by the censoring date (30.1.2020) 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 was re-analyzed with upgraded image analysis pipelines and quality control criteria, which resulted in the exclusion of 10 patients. Therefore, the final post hoc set comprised of 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 (≤10% = low, ≤50% = medium, >50% = high).

Response evaluation

Response evaluation was based on RECIL criteria for lymphoma and ELN criteria for leukemia (42,43). As leukemia patients were not consistently followed up with BM biopsy or aspirate, changes in PB were used equivalently: CR could not be stated with presence of blasts in PB. Analogous to ELN–morphologic leukemia-free state (MLFS), absence of blasts in the peripheral blood without bone marrow available was considered hematological leukemia free state (HLFS) and classified as an objective response (OR). Comparable to morphologic criteria with ELN-partial remission, reduction of blasts in PB by at least 50% was regarded as partial remission (hematological partial remission, HPR=PR). The white blood count (WBC) had to be above 1G/L for HLFS and HPR. Eastern Cooperative Oncology Group performance status (ECOG) 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 to median PFS for a given diagnosis. For T-NHL 9 months of PFS was considered exceptional, for aggressive B-NHL and myeloid disease 18 months were considered exceptional based on the definition by Wheeler et al. (18, 26, 44, 45).

Response assessment was reviewed by an internal study committee consisting of three hematologists (PBS, KM, UJ), a radiologist (MEM), and a pathologist (CK). 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 out of 50 patients showed a PFS of \( \geq 1.3 \). The predefined secondary endpoints were objective response rate (ORR), disease control rate (DCR); progression free survival (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: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing) and MATLAB.

Survival times were compared using the Kaplan-Maier estimator, Log-Rank Tests and Cox-Proportional Hazard Models. Continuous variables were compared Wilcoxon rank sum tests.

A p-value of \(< 0.05\) was considered significant.
References


Figure and Table Legends

Table 1: Characteristics of scFPM-guided patients.

Table 2: Characteristics of exceptional responders

Table 3: Summary of precision medicine trials with comparator (randomized, or PFS ratio, or observational control). # Pt screened – number of patients enrolled or signed the consent form; # Pt tested – number of patients on which test was performed; # Pt with target availability – number of patients in which an actionable target was detected; # Pt assigned to treatment – number of patients with a treatment recommendation; # Pt treated matched – number of patients treated according to treatment recommendation; # Pt 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 was the outcome compared to (control arm, intraindividual benefit, or observational cohort; Study – reference to original study; ASCP - AmpliSeq Cancer Panel; DCR – disease control rate; FISH - fluorescence in situ hybridization; IHC – immunohistochemistry; MALDI-TOF - matrix-assisted laser desorption ionization time-of-flight mass spectrometer; ns – not significant; OS – overall survival; ORR – overall response rate; PFS – progression free survival; Pt – patient; TSACP - TruSeq Amplicon Cancer Panel; TTF - time-to-treatment failure.

Fig. 1: EXALT procedure and primary outcome measure: (A) Viable cells from lymph node (LN), bone marrow (BM), or peripheral blood (PB) of late-stage hematological cancer patients were subjected to image-based scFPM. Target cells are identified by staining with fluorescent antibodies. Reports are automatically generated by the analysis pipeline, are discussed in a dedicated tumor board and patients treated accordingly. (B) Our primary
outcome measure was PFS ratio defined as \( \frac{\text{PFS}_{\text{previous treatment}}}{\text{PFS}_{\text{scFPM treatment}}} \). A ratio of 1.3 is considered individually beneficial.

Fig. 2: CONSORT diagram of study patients

Fig. 3: scFPM-guided treatment enhances PFS ratio in patients with advanced hematological 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 for last previous treatment, stars denote ongoing response for scFPM treatment at the censoring date. PFS ratio is the ratio \( \frac{\text{PFS}_{\text{scFPM treat.}}}{\text{PFS}_{\text{prev. treat}}} \). Below, patient characteristics are color coded and stratified (leukemia versus lymphoma, exceptional response versus non-exceptional response, ECOG>1 versus ECOG≤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 of ≤1 (n=39). Stars denote ongoing response for scFPM treatment at censoring date. (D) Kaplan Meier plot comparing PFS on scFPM treatment between patients with ECOG≤1 (n=39) versus ECOG>1 (n=17). (E) Bar plot showing PFS for all patients with OR on previous treatment. Stars 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 OS stratified according to scFPM-guided (n=56) versus physician’s choice patients (n=20).

Fig. 4: Post Hoc Analysis. (A) Kaplan Meier plot comparing scFPM-matched treatment with previous treatment. Dotted line denotes one year follow up. (B) Kaplan Meier plot comparing non-scFPM-matched treatment with previous treatment. (C) Paired scatter plot comparing non-matched versus matched patients with regards to PFS-ratio. Paired Wilcoxon test.
comparing PFS of previous treatment versus scFPM-matched/non-matched treatment ($H_0$: rank $PFS_{(previous)} = rank PFS_{(current)}$). (D) Kaplan Meier plot of scFPM-matched treatment stratified according to ECOG<1 versus ECOG≥1. (E) Kaplan Meier plot of scFPM-matched treatment stratified according to response on 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%).
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<thead>
<tr>
<th>Table 1</th>
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Table 2

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<td># Pt with target availability</td>
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<tr>
<td>1893</td>
<td>1640 (87%)</td>
<td>341/827 (41%) MALDI-TOF; 583/792 (74%) TSACP; 14/21 (67%) ASCP</td>
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<td>741</td>
<td>520 (70%) sequenced; 638 (86%) IHC; 522 (70%) gene copy number analysis; 496 (67%) complete profile</td>
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<td>500</td>
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<td>106</td>
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<tr>
<td>193</td>
<td>143 (74%)</td>
<td>143/143 (100%)</td>
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</table>
193 screened patients

50 non-eligible patients
- 24 patients with primary endpoint post censoring date
- 11 patients with secondary malignancy
- 7 patients with other treatment available
- 5 patients with PFS last treatment missing
- 3 patients with chronic viral infection

143 tested patients

67 non-evaluable patients
- 21 patients received limited or no treatment
- 20 patients with insufficient material
- 9 patients died early
- 9 patients lost to follow up
- 8 patients without therapy response evaluation

76 evaluated patients

20 physician's choice patients

56 scFPM guided patients

post hoc analysis
Fig. 3

A. All scFPM-guided patients (n = 56)

B. Exceptional responders (n = 12)

C. Patients with ECOG ≤ 1 (n = 39)

D. Patients with ECOG ≤ 1 and ECOG > 1

E. Patients with CR/PR to previous therapy (n = 27)

F. Patients with CR/PR and SD/PD on previous therapy

G. Physician’s choice vs. scFPM

H. All evaluable patients (n = 76)
Functional Precision Medicine Provides Clinical Benefit in Advanced Aggressive Hematological Cancers and Identifies Exceptional Responders

Christoph Kornauth, Tea Pemovska, Gregory I Vladimer, et al.

Cancer Discov  Published OnlineFirst October 11, 2021.

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