Genomics based personalized oncology of cancer of unknown primary

Maximilian Werner[1], Lino Möhrmann[1], Małgorzata Oles[2], Andreas Mock[3], Arne Jahn[1], Simon Kreutzfeldt[3], Sebastian Uhrig[4], Martina Fröhlich[4], Barbara Hutter[4], Daniela Richter[1], Gina Rüter[5], Ivan Jelas[5], Rainer Hamacher[6], Johanna Falkenhorst[6], Sebastian Wagner[7], Christian Brandts[7], Melanie Börries[8], Anna L. Illert[8], Klaus H. Metzeler[9], Benedikt Westphalen[9], Alexander Desuki[10], Thomas Kindler[10], Albrecht Stenzinger[11], Evelin Schröck[12], Peter Horak[3], Christoph Heining[1], Stefan Fröhling[3], Hanno Glimm[1]

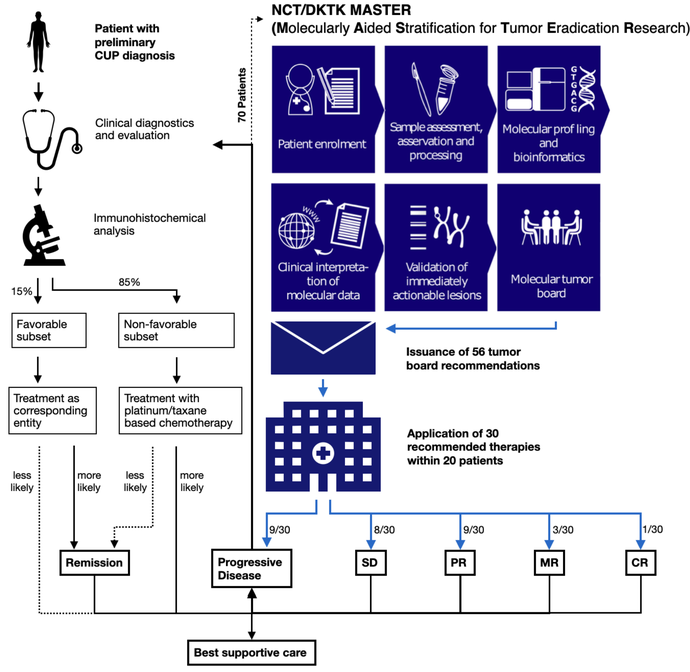
[1] Department of Translational Medical Oncology, National Center for Tumor Diseases (NCT) Dresden and German Cancer Research Center (DKFZ), Dresden, Germany
[2] Translational Functional Cancer Genomics, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center, Heidelberg, Germany
[3] Translational Medical Oncology, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center, Heidelberg, Germany
[4] Division of Applied Bioinformatics, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center, Heidelberg, Germany
[5] Charité Comprehensive Cancer Center, Charité, Berlin, Germany
[6] West German Cancer Center, University Hospital Essen, Essen, Germany
[7] University Cancer Center Frankfurt, University Hospital Frankfurt, Frankfurt, Germany
[8] Comprehensive Cancer Center Freiburg, Freiburg University Medical Center, Freiburg, Germany
[9] Comprehensive Cancer Center München[LMU], Medical Center of the University of Munich, Munich, Germany
[10] University Cancer Center Mainz, University Medical Center Mainz, Mainz, Germany
[11] Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany.
[12] Institute for Clinical Genetics, University Hospital Carl Gustav Carus Dresden, Technical University of Dresden, Dresden, Germany

BACKGROUND

- Cancers of unknown primary site (CUPs) represent a **heterogeneous group of metastatic tumors** and account for three to five percent of all malignancies. They are a frequent cause of cancer death.
- The **median overall survival time is six to ten months** and there are only limited local and systemic treatment options.
- Subgroups of CUP patients representing 15% of all cases benefit from treatment corresponding to the therapy administered to patients with equivalent primary tumors and metastatic spreading.
- Precision oncology is not standard of care for CUP patients.
- Patients in this cohort were discussed in a molecular tumor board and received treatment recommendations based on molecular findings. Follow-up data was analyzed to evaluate treatment outcome of applied recommended therapies.

METHODS

Standard workup



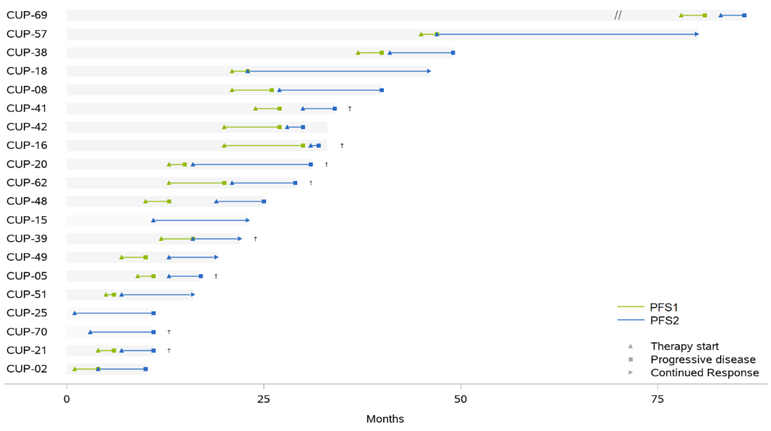
CUP Cohort in MASTER

NCT/DKTK MASTER
(Molecularly **A**ided **S**tratification for **T**umor **E**radication **R**esearch)

- 70 CUP patients were enrolled in a prospective precision oncology registry trial that addresses younger adults with advanced-stage cancer across histologies as well as patients with rare tumors (NCT/DKTK MASTER).
- Molecular analyses performed: Whole genome sequencing (WGS, n=29), whole exome sequencing (WES, n=41) and transcriptome analysis (n=55).
- Clinical analysis included demographic data, histopathological diagnosis, location of metastases at the time of enrollment, fulfillment of the ESMO CUP diagnostic criteria, all systemic therapies and staging information.
- Progression free survival (PFS) of the first treatment based on MASTER (PFS2) was compared to the PFS of the last prior systemic treatment (PFS1) in each individual patient. PFS2/1 ratios (PFSr) were calculated.
- Germline mutations were classified in accordance with ACMG guidelines.

RESULTS

Swimmer plot of 20 patients with applied therapy recommendations



Each bar represents one patient in the study from date of diagnosis until dropout († marks deceased patients) or end of observation period. The last systemic therapy (PFS1, green) and the first recommended therapy (PFS2, blue) are plotted inside those bars. Continued response at the end of the observation period is marked with an arrow. The bar of CUP-69 has been shortened by 60 months (true length 146 months). For CUP-15, CUP-25 and CUP-70 PFSr could not be calculated. 13/17 patients had PFSr ≥ 1.3 (4x checkpoint inhibitor, 3x ALK inhibitor, 3x multikinase inhibitor, 1x trastuzumab, 1x vismodegib, 1x olaparib+gemcitabine).

RESULTS

Cohort description

Characteristics	n
All	70
Sex	
Male	27 (39%)
Female	43 (61%)
Age	
Median (range)	46 (18-73)

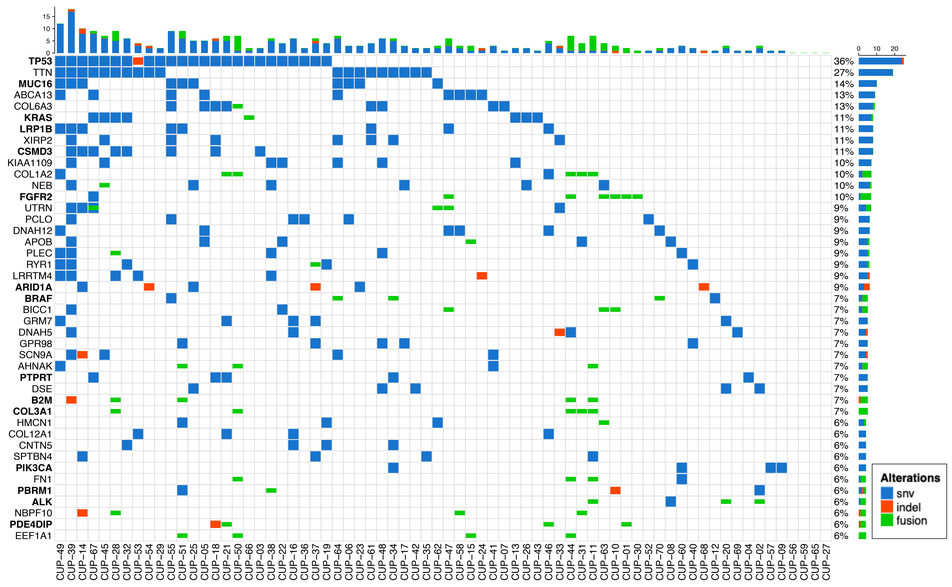
Tissue molecular testing method	
WGS	29
WES	41
RNAseq	55
Molecular tumor board recommendations	
Therapies recommended	56
Therapies applied	20

Clinical outcome

Metric	Value		n	Metric	Value		n
	median (range)	mean			median (range)	mean	
PFS1	89 (31-304) days	110 days	17	prePFS	89 (60-304) days	112 days	17
PFS2	183 (50-805) days	243 days	20	postPFS	720 (50-720) days	448 days	20
PFSr	2.25 (0.16-16.43)	3.61	17	mPFSr	2.67 (0.16-12.20)	4.97	17

We calculated prePFS, postPFS and mPFSr as proposed by Mock et al. (ESMO Open 2019, doi:10.1136/esmoopen-2019-000583).

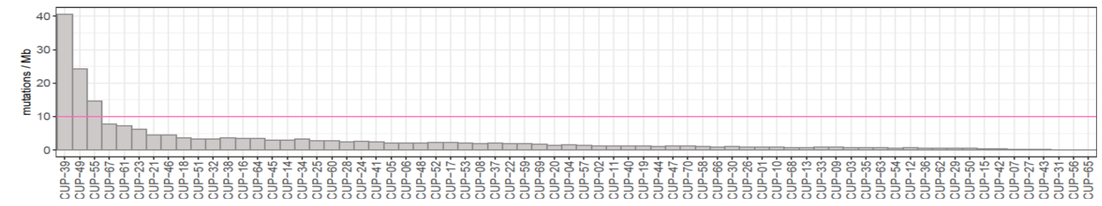
Molecular alterations among 70 CUP patients



Oncoplot: Frequency of nonsynonymous single nucleotide variants (blue), small insertions/deletion (pink) and fusions of high confidence (green) occurring in coding regions of the genes (rows) of individual patients (columns). Only genes mutated in more than 3 patients (while excluding 3 extremely mutated cases with >10 mutations/Mb from counting) appear in the plot. Genes in bold font are listed in the Cosmic Cancer Gene Consensus.

Molecular landscape

DNA: 0 - 1386 SNVs (median, median = 38), 0 - 38 indels (median = 3) per sample within coding sequence. Hypermutation (≥100 SNVs and indels) observed in 18 samples.
CNV-analysis (n=53; 27 WGS / 26 WES) revealed ploidies from two to six (2: n = 33; 3: n = 6; 4: n = 11; 6: n = 3), and complex CNV profiles in most cases. Events occurring in more than 40% of samples included: gains in chromosomes 8q, 1q and 7 (with maxima in 55%, 52% and 45% of the samples in cytobands q24.13, q41, p22.2, respectively), losses in chromosomes 6q and 17p (with maxima in 46% and 43% of the samples in cytobands q23.3 and p13.1, respectively). Only one sample showed a normal CNV profile.
RNA: 0 to 61 (median = 10) gene fusions of high confidence per patient. The most common fusion involved the FGFR2 gene (n = 6), always occurring at the splice-site of the FGFR2 gene, in four cases intrachromosomal including inversions (n=3, twice with BICC1 gene), translocations (n=2) and one deletion (n=1). EML4-ALK fusions were detected in 3 patients. We found several rare fusions, including a MXI1-NUTM1 fusion which led the MTB to recommend pathological reevaluation since NUTM1 fusions define NUT midline carcinomas.
Diagnostic reevaluation: Based on characteristic genetic events the MTB recommended reevaluation by pathologists in 7 cases.
Germline analysis of all 70 patients revealed six pathogenic variants or likely pathogenic variants in five patients. Affected genes were CHEK2, BRCA1, CDKN2A, FH, NBN and ERCC3. Genetic counseling was recommended.



Tumor mutational burden: Number of non-synonymous somatic mutations in exonic sequences (SNVs and small insertions/deletions) per megabase (Mb) of coding sequence of the genome. 3 samples above 10 mut/Mb threshold (pink line) were identified as ultra-hypermutated and were excluded from the pool of mutations considered for thresholding in the oncoplot.

CONCLUSIONS AND PERSPECTIVES

I. **A comprehensive molecular analysis of CUPs provides clinically relevant information and additional, molecularly stratified treatment options, which can be highly beneficial even in heavily pretreated patients.**

II. **Further investigation in larger cohorts using innovative prospective trial designs is needed.**

NCT
NATIONAL CENTER FOR TUMOR DISEASES
PARTNER SITE DRESDEN
UNIVERSITY CANCER CENTER UCC

Supported by:
German Cancer Research Center
University Hospital Carl Gustav Carus Dresden
Carl Gustav Carus Faculty of Medicine, TU Dresden
Helmholtz-Zentrum Dresden-Rossendorf

E-Mail: maximilian.werner@nct-dresden.de