BACKGROUND

- Cancers of unknown primary site (CUPs) represent a heterogeneous group of metastatic tumours 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 tumours and metastatic spreading.
- Precision oncology is not standard of care for CUP patients.
- Patients in this cohort were discussed in a molecular tumour 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

- 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 tumours (NCT00791636, MASTERCUP).
- 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 enrolment, fulfillment of the ESMO-Molecular guidance criteria, all systemic therapies and staging information.
- Progression free survival (PFS) of the first treatment based on MASTERCUP was compared to the PFS of the last prior systemic treatment (PFS1) in each individual patient. PFS1/PFS (ratio PFS) were calculated.
- Germline mutations were classified in accordance with ACMG guidelines.

RESULTS

Cohort description

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>Tissue molecular testing method</th>
<th>Metric</th>
<th>Value</th>
<th>n</th>
<th>Metric</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>70</td>
<td>WGS</td>
<td>median (range)</td>
<td>mean</td>
<td>29</td>
<td>median (range)</td>
<td>mean</td>
</tr>
<tr>
<td>Male</td>
<td>43 (61%)</td>
<td>WES</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>27 (39%)</td>
<td>RNAseq</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular tumour board recommendations</td>
<td></td>
<td>Therapies recommended</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Median (range)</td>
<td>46 (18-73)</td>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Clinicopathological parameters

- Overall Survival (OS): We calculated PFS1, postPFS and mPFSR as proposed by Mock et al. (ESMO Open 2019, doi:10.1136/esmoopen-2019-000583).

Molecular alterations among 70 CUP patients

- DNA: 0 - 1386 SNVs (median, median = 38), 0 - 38 indels (median = 3) per sample within coding sequence. Hypomutation (<10 SNVs and indels) observed in 18 samples.
- CNV-analysis (n=53): 27 WGS / 26 WES revealed ploidy from two to six (n = 33; n = 6; n1/n2/n3 = 11; n6 = 3), and complex CNVs in most cases. Events occurring in more than 40% of samples included: gains in chromosomes 8q, 15q, and 7p; losses in the genome (720) to 15p (720) days (median = 6). Gains of 4q13, 4q14, 1p22, respectively.
- Losses in chromosomes 6q and 1p due to (median = 6) and 4% of the samples in cytobands q23.3 and p13.1, respectively.
- Only one sample showed a normal CNV profile: 0.

Oncoplot: Frequency of non-synonymous single nucleotide variants (blue), small insertions/deletions (pink) and fusions (large red arrows) 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.

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.

Molecular landscape

- DNA: 0 - 1386 SNVs (median, median = 38), 0 - 38 indels (median = 3) per sample within coding sequence. Hypomutation (<10 SNVs and indels) observed in 18 samples.
- CNV-analysis (n=53): 27 WGS / 26 WES revealed ploidy from two to six (n = 33; n = 6; n1/n2/n3 = 11; n6 = 3), and complex CNVs in most cases. Events occurring in more than 40% of samples included: gains in chromosomes 8q, 15q, and 7p; losses in the genome (720) to 15p (720) days (median = 6). Gains of 4q13, 4q14, 1p22, respectively.
- Losses in chromosomes 6q and 1p due to (median = 6) and 4% of the samples in cytobands q23.3 and p13.1, respectively.
- Only one sample showed a normal CNV profile: 0.

Oncoplot: Frequency of non-synonymous single nucleotide variants (blue), small insertions/deletions (pink) and fusions (large red arrows) 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.

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.

Molecular landscape

- DNA: 0 - 1386 SNVs (median, median = 38), 0 - 38 indels (median = 3) per sample within coding sequence. Hypomutation (<10 SNVs and indels) observed in 18 samples.
- CNV-analysis (n=53): 27 WGS / 26 WES revealed ploidy from two to six (n = 33; n = 6; n1/n2/n3 = 11; n6 = 3), and complex CNVs in most cases. Events occurring in more than 40% of samples included: gains in chromosomes 8q, 15q, and 7p; losses in the genome (720) to 15p (720) days (median = 6). Gains of 4q13, 4q14, 1p22, respectively.
- Losses in chromosomes 6q and 1p due to (median = 6) and 4% of the samples in cytobands q23.3 and p13.1, respectively.
- Only one sample showed a normal CNV profile: 0.

Oncoplot: Frequency of non-synonymous single nucleotide variants (blue), small insertions/deletions (pink) and fusions (large red arrows) 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.

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.