Supplementary Information

The patterns and dynamics of genomic instability in metastatic pancreatic cancer
Peter J Campbell; Shinichi Yachida; Laura J Mudie; Philip J Stephens; Erin D Pleasance; Lucy A Stebbings; Laura A Morsberger; Calli Latimer; Stuart McLaren; Meng-Lay Lin; David J McBride; Ignacio Varela; Serena A Nik-Zainal; Catherine Leroy; Mingming Jia; Andrew Menzies; Adam P Butler; Jon W Teague; Constance A Griffin; John Burton; Harold Swerdlow; Michael A Quail; Michael R Stratton; Christine Iacobuzio-Donahue; P Andrew Futreal.

Table of contents

Supplementary results
  Effects of rearrangements on protein-coding genes
  Fold-back inversions
  Patterns and dynamics of genomic amplification
  Signatures of DNA repair
  References
  Supplementary figure legends
  Supplementary figure 1
  Supplementary figure 2
  Supplementary figure 3
  Supplementary figure 4
  Supplementary figure 5
  Supplementary figure 6
  Supplementary figure 7
  Supplementary figure 8
  Supplementary figure 9
  Supplementary figure 10
  Supplementary figure 11
  Supplementary table legends
  Supplementary table 4
  Supplementary table 5
Supplementary results

Effects of rearrangements on protein-coding genes

Genomic instability drives cancer development through effects on genes, such as copy number changes, internal deletion or duplication of exons within a gene, fusion of coding exons from two genes into a novel open reading frame or juxtaposition of an intact gene with regulatory elements of another gene\(^1\). We found several examples of such events among the pancreatic cancer rearrangements (supplementary table 5). These included deletions of known tumour suppressor genes in pancreatic cancer, including \textit{CDKN2A} (5 patients), \textit{SMAD4} (5) and \textit{TP53} (1), as well as amplifications of the cancer genes \textit{MYC} (4 patients), \textit{KRAS} (1) and \textit{CCNE1} (1).

Ten rearrangements fused portions of two genes together in the same orientation, of which five were predicted to be in-frame (supplementary figure 3, supplementary table 5). No gene fusions were recurrent in the 13 samples or known to cause pathogenic rearrangements in other cancer types\(^2\), and the overall fraction of 10 fusions/381 rearrangements is about what would be expected by chance. Approximately a third of the genome is located in a gene footprint, hence the chances of both ends of a breakpoint occurring in a gene is 1/9. For a fusion gene to result, the two genes must be in the same orientation, further halving the odds. Finally, the two genes must be in the same phase of the reading frame, which has a probability of about 1/2 once splice variants are considered. Thus, roughly 1/36 randomly positioned rearrangements would generate an in-frame fusion gene by chance, very similar to the proportion
actually observed (1/38). This suggests that most of the fusions observed are probably chance events, not driving cancer biology.

There were, however, genes with recurrent internal exon rearrangements. Most frequent among these was MACROD2 with four exonic deletions, three of which were in-frame, and a further seven deletions confined to introns (supplementary figure 4). There was remarkable clustering of deletions within patients, including one patient with five separate deletions in intron 5. These features suggest that MACROD2 resembles a chromosomal fragile site. Indeed, other fragile sites were recurrently rearranged, including PARK2 and WWOX, although the recent observation that PARK2 may be a tumour suppressor gene underlines that fragile sites may harbour cancer genes.

Several other samples were found with potentially interesting rearrangements. PD3639 carried a homozygous, in-frame deletion of exon 2 of PARD3 (supplementary figure 3F), thought to be a master regulator of apical-basal cell polarity. Interestingly, we and others have found clusters of homozygous microdeletions in this gene in cancer samples, and functional studies have revealed that restoring the gene to cell lines with deletions restores tight junctions and reduces contact-dependent proliferation. Also of interest is the cluster of rearrangements on 18q around the RIOK3 gene found in patient PD3645. This gene is a kinase amplified in ~16% of pancreatic cancers. It has diverse functional roles affecting cytoskeletal structure, but can importantly increase pancreatic ductal cell motility and invasion. We also found a patient
with rearrangements increasing copy number of another recurrently amplified gene on chromosome 18q, GATA6, as discussed in much greater detail below.

The genes involved at each breakpoint in the set of 381 somatically acquired rearrangements are documented in detail in supplementary table 6.

**Fold-back inversions**

We believe breakage-fusion-bridge cycles to be the most likely mechanism for generating fold-back inversions, although two other mechanisms could also potentially explain the observed genomic structure. Firstly, stalled replication forks during S-phase could stimulate template switching\(^8\), in which the new strand being synthesised meets a block in its progress and invades the opposite strand nearby and continues extension in the reverse direction. This model is termed FoSTeS (Fork Stalling and Template Switching)\(^8\). Secondly, inverted duplications could also cause this genomic structure at the breakpoint, as we have seen previously\(^9\). Overall, however, we favour breakage-fusion-bridge cycles as the likely explanation. In particular, end-to-end fusion of chromosomes has been directly observed in cytogenetic preparations of pancreatic cancer cells\(^10\). Furthermore, we observed three fold-back inversions in which 100-250bp fragments of genomically distant DNA were captured at the breakpoint (supplementary figure 6A). One was a fragment of centromeric repeat, one a fragment of the same chromosome from 38Mb away and one a fragment of a different chromosome. Although theoretically possible, it seems unlikely that stalled replication forks would switch templates to such geographically distant regions before switching back to virtually the identical starting point. The major
reason that we do not favour inverted duplications as the mechanism for fold-back inversions is that we do not find evidence for the reciprocal breakpoints required for an inverted duplication.

Interestingly, breakage-fusion-bridge cycles would predict identical DNA ends to be joined. In fact, the two ends map to the reference genome a few hundred to thousand base-pairs apart (supplementary figure 6B). This quantifies the problem of chromosome end-replication in the absence of telomeres, implying that the inability to complete lagging strand DNA synthesis during S-phase leads to the loss of 100s-1000s of base-pairs from the chromosome end per cell cycle.

**Patterns and dynamics of genomic amplification**

Three mechanisms of amplification are apparent in the pancreatic cancer data, each with distinct genomic structure and dynamics. A $\textit{KRAS}$ amplicon in PD3642 (supplementary figure 7A) shows a complex network of back-and-forth rearrangements, predominantly intrachromosomal, with several crossing the centromere, occurring in both inverted and non-inverted orientations. This pattern suggests a model in which rearrangements occur sporadically over multiple cell cycles. Those that amplify the mutated $\textit{KRAS}$, even if by only 1-2 copies, confer a selective advantage to the clone. Genomic regions incorporated into the developing amplicon early may be co-amplified by subsequent rearrangements. Such a process would create the ruckled copy number profile across the region we observe, although the co-amplified regions themselves do not necessarily confer additional selective advantage to the cancer. Under this model, copies of the target gene need not be geographically localised:
rearrangements with chromosomes 11 and 14, for example, suggest that copies of \textit{KRAS} may be carried on several chromosomes.

We observed signatures of extrachromosomal amplification in one sample (supplementary figure 7B). In addition to \textit{MYC} amplification, the patient had two distinct amplicons on chromosome 18 and one on X. In contrast to the \textit{KRAS} amplicon discussed above, those on 18 and X show sharp increase in copy number from baseline to 5-10 copies at either end, with little change in copy number within each region. Three interlinking rearrangements trace a path through the three amplicons, and imply that they originated as episomal circles of DNA at 5-10 copies/cell (supplementary figure 7C). A rearrangement linking the amplicon on X to the \textit{MYC} region may represent a locus at which the episomes underwent serial tandem insertion into genomic DNA. This insight into rearrangement dynamics suggests that the chromosome 18 and X episome was amplified separately to the \textit{MYC} amplification before a late conjunction of the two. Therefore, unlike the co-amplified regions in the \textit{KRAS} amplicon above, the episome is itself likely to contain a second target gene. \textit{GATA6} is the most likely candidate here, recently found to be amplified in 10-15\% of pancreatic cancers\textsuperscript{11,12}.

The third pattern of genomic amplification we observed is of multiple fold-back inversions, exemplified by an amplicon on chromosome 8q in patient PD3641 (supplementary figure 7D). The end-to-end fusion of sister chromatids generates a dicentric chromosome that is pulled to both daughter cells (anaphase bridge). The completion of mitosis inevitably creates another chromosome breakage, and
the breakage-fusion-bridge process can repeat in each subsequent cell cycle until the chromosome acquires a genomic segment capped by telomeres. This process can rapidly amplify genomic segments, as evidenced by the example shown in which 16-fold amplification resulted from just 5 cycles of breakage-fusion-bridge before possibly resolving by insertion into chromosome 8p. Interestingly, the patient in whom we observed this breakage-fusion-bridge amplicon on chromosome 8q showed a large number of rearrangements involving the chromosome, but very few elsewhere in the genome (see figure 1B, main paper). We hypothesise that some time during its evolution, this clone of cancer cells has experienced a focal genomic instability involving chromosome 8. This might have been tipped off by localised telomere attrition which then initiated a series of breakage-fusion-bridge events. Within a few cell cycles, significant numbers of dsDNA breaks and genomic rearrangements could result, restricted to this single chromosome.

Rearrangements that occur early in amplicon genesis are themselves copied by subsequent rearrangements, manifesting as a breakpoint spanned by many reads demarcating a large change in copy number. On this basis, fold-back inversions are often early or even initiating rearrangements for amplification of cancer genes in pancreatic cancer (supplementary figure 8A-B), even though subsequent rearrangements may be more of the back-and-forth pattern with ruckled copy number described above. Thus, given that breakage-fusion-bridge cycles may be initiated by telomere erosion, this is supportive evidence for the theory that telomere dysfunction plays a critical early role in the genomic instability of pancreatic cancer$^{13,14}$. 
Signatures of DNA repair

Annotation of rearrangements to base-pair resolution enables profiling of DNA joins, reflecting mechanisms of DNA repair (supplementary figure 9A). We find that in 61% of pancreatic cancer rearrangements, the two ends join at stretches of 1-6bp of identical sequence, known as microhomology. A further 19% have a few bases of non-templated sequence inserted between the two genomic ends, and the remaining 20% have neither microhomology nor non-templated sequence at the junction. The distribution is bimodal with separate peaks at 0bp and 2bp of microhomology. This suggests that at least two pathways are operative in the (incorrect) repair of DNA breaks in pancreatic cancer, one using microhomology-mediated sequences and one a direct end-joining process.

There are striking differences in sequence homology at breakpoints across different classes of rearrangement (p<0.001; supplementary figure 9B). Deletions and tandem duplications show predominance of microhomology-mediated repair, with a strong peak at 2bp sequence homology. In contrast, fold-back inversions and amplicon-related rearrangements show a bimodal pattern with approximately equal balance between microhomology-mediated and direct end-joining pathways, whereas interchromosomal rearrangements favour direct end-joining. Intriguingly, these patterns mimic those seen in breast cancer across the different classes of rearrangement. This suggests that, despite marked differences in the frequency of rearrangement categories between the two cancer types, the mechanisms of DNA repair operative in each category are broadly similar.
References

Supplementary figure legends

**Supplementary figure 1.** Flow-chart showing the samples analysed and the molecular and informatic pathways used to annotate the cancer genomes. T, tumour; N, normal.

**Supplementary figure 2.** Number of paired reads generated per sample, and the results achieved with the mapping algorithm.

**Supplementary figure 3.** Fusion genes. (A) In PD3637, an inversion with large deletions at both reciprocal breakpoints was observed. One breakpoint joined the 5’ portion of SYNE1 to the 3’ portion of MTHFD1L. (B) Also in PD3637, an inverted rearrangement on chromosome 22 fuses exons 1-4 of KIAA1671 to exons 4-6 of XBP1. (C) In PD3640, a small ~80kb apparent deletion (although without major copy number change) on chromosome 1 fuses the genes USP48 to RAP1GAP. (D) An unbalanced translocation between chromosomes 17 and 18 in PD3644 generates a potential in-frame fusion gene between SPECC1 and ZNF532. Interestingly, the copy number loss on chromosome 18 resulting from this unbalanced translocation is responsible for deleting one copy of SMAD4. (E) An unbalanced translocation between chromosomes 4 and 14 in PD3646 generates a potential in-frame fusion gene comprising the 5’ end of ANK2 and the 3’ end of KCNK10. (F) A homozygous deletion in PD3639 removes exon 2 of PARD3 while preserving the reading frame of the gene.
**Supplementary figure 4.** Clusters of deletions in *MACROD2*. Four of 13 samples carried deletions in *MACROD2*, including PD3644 with three overlapping in-frame exonic deletions and PD3827 with five separate deletions all in intron 5. The lack of a well-defined minimally deleted region coupled with other genomic features imply that this cluster of deletions has arisen more from chromosomal fragility than through positive selection pressure.

**Supplementary figure 5.** Circle plots showing the genomic landscape of rearrangements in samples for which plots are not already shown in figure 1B. Chromosome ideograms are shown around the outer ring with copy number plots on the inner ring. Individual rearrangements are shown as arcs joining the two genomic loci, each coloured according to the type of rearrangement.

**Supplementary figure 6.** Genomic patterns of fold-back inversions. (A) Some fold-back inversions captured fragments of templated genomic DNA between the two ends. These were often from distant regions of the genome, such as centromeric repeats or adjacent to other dsDNA breaks involved in somatic rearrangements. (B) Histogram showing the distance between the two inverted ends in the set of fold-back inversions.

**Supplementary figure 7.** Patterns of genomic amplification. (A) Back-and-forth amplification with ruckled copy number is exemplified by a *KRAS* amplicon in patient PD3642. Numerous rearrangements, in both inverted and non-inverted orientation, some spanning the centromere (at ~35Mb), some interchromosomal, many demarcating copy number changes, combine to amplify
the target gene. In this process, rearrangements occur sequentially such that distinct regions picked up in early events are themselves co-amplified by subsequent rearrangements, leading to the characteristic up-and-down pattern of copy number changes observed. (B) Extrachromosomal amplification is implied by the sharp copy number changes on chromosomes 18 (twice) and X demarcated by three rearrangements through which a circular path can be tracked. There is also a rearrangement joining the chromosome X segment to a MYC amplicon, which may represent a site at which extrachromosomal circles tandemly inserted into the genome. (C) Structure of the episomal circle suggested by the patterns of rearrangement shows an intact GATA6 gene, the likely target of amplification. (D) An amplicon on chromosome 8q from PD3641 derives from a cluster of fold-back inversions. This could have arisen in as short a time as five cell cycles, each associated with one breakage-fusion-bridge cycle, before being resolved by acquisition of a genomic segment with a telomere (possibly from the 8p12 rearrangements).

**Supplementary figure 8.** Fold-back inversions as key early events in amplifications of cancer genes. (A) The MYC amplicon of PD3641 was probably initiated by a fold-back inversion downstream of MYC (marked with *), since this rearrangement demarcates a change from normal copy number to the most heavily amplified region of the amplicon. Subsequent evolution of the amplicon shows more the back-and-forth pattern of intrachromosomal rearrangements with ruckled copy number exemplified in figure 2A, with most of the breaks occurring upstream of MYC. This suggests that the entire cassette containing two copies of MYC in inverted orientation to one another was itself amplified by
subsequent rearrangements. (B) The CCNE1 amplicon of PD3827 contains several fold-back inversions, many of which are likely to have been early events in the amplification. In particular, the rearrangement marked with * demarcates normal copy number from the most heavily amplified region of chromosome 19, and was spanned by 93 reads. It is therefore likely to represent one of the earliest events in the amplicon’s development.

Supplementary figure 9. Signatures of DNA repair at the breakpoint. (A) Patterns of microhomology (teal), non-templated sequence (red) or direct end-joining (yellow) in the pancreatic cancer rearrangements. (B) Comparison of patterns of microhomology and non-templated sequence across different categories of rearrangement between breast (yellow) and pancreatic (green) cancer.

Supplementary figure 10. Rearrangements found in all metastases of PD3637 but not the primary tumour demonstrate geographic co-localisation (red; chromosomes 14, 16 and 21), suggesting they originated from DNA breaks acquired in a short period of time.

Supplementary figure 11. Patterns of rearrangements and phylogenetic relationships observed in PD3828. (A) Copy number and rearrangements on chromosome 8 for a lung metastasis from PD3828 show increase copy number focused around MYC caused by multiple rearrangements. (B) Flat, diploid copy number and lack of rearrangements for the same region of chromosome 8 in a
diaphragm metastasis from the same patient, as analysed by massively parallel, paired-end sequencing of this metastasis.
Massively parallel, paired-end sequencing
17 samples (13 patients)
~65,000,000 paired reads / sample
Read-length: 37bp paired-end
DNA fragments: 400-500bp
Aligned against reference genome

Clusters of potential rearrangements or those near copy number change

Filters:
Multiple independent reads
+/- Near copy number change
Not <1Mb from seq gap
Not known germline variant
Confirmatory PCR & Seq
T & N genomes
PCR for somatically acquired rearrangements across other metastases from that individual (10 patients).

Counts per genomic window containing constant amount of mappable sequence
Circular binary segmentation

Filters:
>3 bins / segment
Allelic number estimated from bin counts

13 patients with adenocarcinoma of the pancreas
3 patients: Primary tumour sequenced
8 patients: Single metastasis sequenced
Other metastases PCR genotyped
2 patients: Three metastases sequenced
Other metastases PCR genotyped

3 patients:
8 patients:
2 patients:
3 patients:
8 patients:
2 patients:
MACROD2 deletions

Exons 1–2 3 4 5 6 7 8 9–17

Chr 20:
13,924,410

Chr 20:
15,981,842

- In-frame
- Out-of-frame
- Intronic

PD3644
PD3640
PD3639
PD3827

Supplementary figure 4
Supplementary figure 5

Inversion
Deletion
Tandem duplication
Fold-back inversion
Amplicon
Interchromosomal
Other intrachromosomal
A. **PD3641 MYC amplicon**

- Head-to-head
- Tail-to-tail
- Tandem dup-type
- Deletion-type
- Fold-back inversion

B. **PD3827 CCNE1 amplicon**

- CCNE1

**Chromosome 8 genomic position (Mb)**

**Chromosome 19 genomic position (Mb)**

*Supplementary figure 8*
Omnipresent
All mets, not primary
} Partially shared
A  PD3828 lung metastasis

- Red: Head-to-head
- Blue: Tail-to-tail
- Teal: Tandem dup-type
- Brown: Deletion-type
- Purple: Fold-back inversion

MYC

Genomic position chromosome 8 (Mb)

B  PD3828 diaphragm metastasis
**Supplementary table legends**

**Supplementary table 1.** Clinical and pathology characteristics of patients and samples studied by massively parallel, paired-end sequencing

**Supplementary table 2.** Somatically acquired genomic rearrangements in 13 patients with pancreatic cancer. All structural variants have been confirmed by PCR across the breakpoint, with bidirectional sequencing confirming the segments involved. Most have had the breakpoint annotated to base-pair resolution ('Seq' in the Evidence column): for the others, we provide a range of genomic positions encompassing the breakpoints ('PCR across bkpt'). Length and sequence of either microhomology or non-templated sequence at the junction are shown.

**Supplementary table 3.** Germline genomic rearrangements in 13 patients with pancreatic cancer. All structural variants have been confirmed by PCR across the breakpoint, with bidirectional sequencing confirming the segments involved. Length and sequence of either microhomology or non-templated sequence at the junction are shown.

**Supplementary table 4.** Nomenclature, classification and definitions of genomic rearrangements.

**Supplementary table 5.** Rearrangements associated with internally rearranged genes, fusion genes or copy number changes of known cancer genes.
Supplementary table 6. Genes involved at both breakpoints for each of the somatically acquired genomic rearrangements.

Supplementary table 7. Presence or absence of each somatically acquired genomic rearrangement across the available metastasis and primary tumour samples for 10 patients (1 = present by PCR; 0 = absent by PCR).
**Supplementary table 4.** Nomenclature, classification and definitions of genomic rearrangements.

A given rearrangement is classified according to (i) whether it occurs in an amplicon; (ii) whether it is interchromosomal or intrachromosomal (if not in an amplicon); and (iii) for an intrachromosomal rearrangement, whether it is a deletion, tandem duplication, inversion, fold-back inversion or other, more complex pattern.

1. **Intrachromosomal rearrangement**
   a. **Deletion**
      - Requires copy number decrease to be evident if end-points are further apart than lower limit of resolution of copy number analysis (200kb for this paper).
      - One breakpoint only
   
   b. **Tandem duplication**
      - Requires copy number change increase to be evident if end-points are further apart than lower limit of resolution of copy number analysis
      - One breakpoint only
   
   c. **Inversion**
      - Copy number neutral (unless associated with sub-deletion / duplications at the breakpoint)
      - Two breakpoints required – in the absence of sub-deletions or duplications these should be at the same genomic locations but opposite directions (ie one head-to-head and one tail-to-tail). If only one breakpoint identified, label as ‘Intrachromosomal rearrangement with inverted orientation’
   
   d. **Fold-back inversion**
      - Requires a single inverted rearrangement without evidence for the reciprocal partner of a straightforward inversion (category c).
      - Must demarcate a copy number change.
      - The two ends of the breakpoint must be separated by <20kb.
   
   e. **Intrachromosomal rearrangement**
      - Can be sub-classified as in non-inverted or inverted orientation.
      - For this paper, inverted duplications are included in this category.
      - Includes those rearrangements which cannot be robustly classified on the basis of copy number data and other associated rearrangements in any of the categories above.

2. **Interchromosomal rearrangement**

3. **Rearrangements involving amplicons**
a. Intrachromosomal amplicon-to-amplicon

b. Intrachromosomal amplicon-to-nonamplified DNA

c. Interchromosomal amplicon-to-amplicon

d. Interchromosomal amplicon-to-nonamplified DNA

e. Fold-back inversion (defined as in category 1d above).

4. Insertion of non-templated sequence
Supplementary table 5. Rearrangements associated with internally rearranged genes, fusion genes or copy number changes of known cancer genes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rearrangement</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Predicted in-frame fusion genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD3637</td>
<td>Inversion</td>
<td>Fuses exons 1-25 of SYNE1 to exons 27-28 of MTHFD1L in frame</td>
</tr>
<tr>
<td>PD3637</td>
<td>Inverted duplicatë</td>
<td>Fuses exons 1-4 of KIAA1671 to exons 4-6 of XBP1 in frame</td>
</tr>
<tr>
<td>PD3640</td>
<td>Deletion</td>
<td>Fuses exons 1-22 of USP48 to exons 2-19 of RAP1GAP in frame</td>
</tr>
<tr>
<td>PD3644</td>
<td>Interchromosomal</td>
<td>Fuses exons 1-12 of SPECC1 to exons 5-11 of ZNF532 in frame</td>
</tr>
<tr>
<td>PD3646</td>
<td>Interchromosomal</td>
<td>Fuses exons 1-10 of ANK2 to exons 5-7 of KCNK10 in frame</td>
</tr>
<tr>
<td><strong>Predicted out-of-frame fusion genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD3638</td>
<td>Inverted intrachr.</td>
<td>Fuses exon 1 of FLJ43752 to exon 2 of OR10C1 out of frame</td>
</tr>
<tr>
<td>PD3638</td>
<td>Interchromosomal</td>
<td>Fuses exons 1-2 of C6ORF138 to exons 7-12 of SLC1A6 out of frame</td>
</tr>
<tr>
<td>PD3642</td>
<td>Non-inverted intra.</td>
<td>Fuses exons 1-2 of ENO1 to exons 4-10 of CAPZB out of frame</td>
</tr>
<tr>
<td>PD3644</td>
<td>Deletion</td>
<td>Fuses exon 1 of XPO7 to exons 4-14 of DPYSL2 out of frame</td>
</tr>
<tr>
<td>PD3828</td>
<td>Inverted intrachr.</td>
<td>Fuses exons 1-4 of ZNF7 to exons 4-6 of ZMAT4 out of frame</td>
</tr>
<tr>
<td><strong>Predicted in-frame internal gene rearrangements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD3639</td>
<td>Deletion</td>
<td>In-frame deletion of exon 2 of PARD3 (homozygous)</td>
</tr>
<tr>
<td>PD3644</td>
<td>Deletion</td>
<td>In-frame deletion of exon 4 of MACROD2</td>
</tr>
<tr>
<td>PD3644</td>
<td>Deletion</td>
<td>In-frame deletion of exon 4 of MACROD2</td>
</tr>
<tr>
<td>PD3645</td>
<td>Deletion</td>
<td>In-frame deletion of exons 3-5 of MACROD2</td>
</tr>
<tr>
<td>PD3827</td>
<td>Deletion</td>
<td>In-frame deletion of exons 8-9 of GMDS</td>
</tr>
<tr>
<td>PD3828</td>
<td>Deletion</td>
<td>In-frame deletion of exon 2 of A2BP1</td>
</tr>
<tr>
<td>PD3640</td>
<td>Tandem duplicatë</td>
<td>In-frame duplication of exons 14-32 of CSMD3</td>
</tr>
<tr>
<td>PD3826</td>
<td>Tandem duplicatë</td>
<td>In-frame duplication of exon 4 of EEPD1</td>
</tr>
<tr>
<td>PD3827</td>
<td>Tandem duplicatë</td>
<td>In-frame duplication of exons 2-5 of PKP2</td>
</tr>
<tr>
<td><strong>Predicted out-of-frame internal gene rearrangements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD3640</td>
<td>Deletion</td>
<td>Out-of-frame deletion of exon 11 of PRPSAP2</td>
</tr>
<tr>
<td>PD3640</td>
<td>Deletion</td>
<td>Out-of-frame deletion of exon 6 of MACROD2</td>
</tr>
<tr>
<td>PD3640</td>
<td>Deletion</td>
<td>Out-of-frame deletion of exon 6 of PARK2</td>
</tr>
<tr>
<td>PD3640</td>
<td>Deletion</td>
<td>Out-of-frame deletion of exons 3-4 of NPAR3</td>
</tr>
<tr>
<td>PD3827</td>
<td>Deletion</td>
<td>Out-of-frame deletion of exons 4-5 of FAM190A</td>
</tr>
<tr>
<td>PD3827</td>
<td>Deletion</td>
<td>Out-of-frame deletion of exon 6 of PARK2</td>
</tr>
<tr>
<td>PD3827</td>
<td>Deletion</td>
<td>Out-of-frame deletion of exon 6 of PARK2</td>
</tr>
<tr>
<td>PD3827</td>
<td>Deletion</td>
<td>Out-of-frame deletion of exons 7-10 of PTK2</td>
</tr>
<tr>
<td>PD3827</td>
<td>Deletion</td>
<td>Out-of-frame deletion of exon 2 of NRG3</td>
</tr>
<tr>
<td>PD3639</td>
<td>Tandem duplicatë</td>
<td>Out-of-frame duplication of exons 7-8 of WWOX</td>
</tr>
<tr>
<td>PD3643</td>
<td>Tandem duplicatë</td>
<td>Out-of-frame duplication of exons 12-13 of PTPRZ1</td>
</tr>
<tr>
<td><strong>Predicted promoter fusions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD3637</td>
<td>Inverted intrachr.</td>
<td>Places intact EID2B into upstream region of IL29</td>
</tr>
<tr>
<td>PD3643</td>
<td>Deletion</td>
<td>Places intact CLDN12 into upstream region of STEAP2</td>
</tr>
<tr>
<td>PD3645</td>
<td>Non-inverted intra.</td>
<td>Places intact MOCOS into upstream region of RIOK3</td>
</tr>
<tr>
<td>PD3646</td>
<td>Interchromosomal</td>
<td>Places intact AADACL3 into upstream region of KIAA1217</td>
</tr>
<tr>
<td>PD3827</td>
<td>Amplicon</td>
<td>Places intact SIPAIL3 into upstream region of GNG7</td>
</tr>
</tbody>
</table>
### Rearrangements affecting copy number of known cancer genes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Event Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD3638</td>
<td>Deletion</td>
<td>Heterozygous deletion of <strong>CDKN2A</strong></td>
</tr>
<tr>
<td>PD3640</td>
<td>Deletion</td>
<td>Heterozygous deletion of <strong>CDKN2A</strong></td>
</tr>
<tr>
<td>PD3641</td>
<td>Deletion x2</td>
<td>Compound heterozygous deletion of <strong>CDKN2A</strong></td>
</tr>
<tr>
<td>PD3644</td>
<td>Deletion</td>
<td>Heterozygous deletion of <strong>CDKN2A</strong></td>
</tr>
<tr>
<td>PD3645</td>
<td>Deletion</td>
<td>Heterozygous deletion of <strong>CDKN2A</strong></td>
</tr>
<tr>
<td>PD3640</td>
<td>Deletion</td>
<td>Heterozygous deletion of <strong>TP53</strong></td>
</tr>
<tr>
<td>PD3637</td>
<td>Complex intrachr.</td>
<td>Homozygous loss of <strong>SMAD4</strong></td>
</tr>
<tr>
<td>PD3640</td>
<td>Complex intrachr.</td>
<td>Heterozygous loss of <strong>SMAD4</strong></td>
</tr>
<tr>
<td>PD3643</td>
<td>Unbalanced transloc.</td>
<td>Heterozygous loss of <strong>SMAD4</strong></td>
</tr>
<tr>
<td>PD3644</td>
<td>Unbalanced transloc.</td>
<td>Homozygous loss of <strong>SMAD4</strong> (some rearrangements not identified)</td>
</tr>
<tr>
<td>PD3645</td>
<td>Complex intrachr.</td>
<td>Heterozygous loss of <strong>SMAD4</strong></td>
</tr>
<tr>
<td>PD3640</td>
<td>Amplification</td>
<td>Amplification of <strong>MYC</strong></td>
</tr>
<tr>
<td>PD3641</td>
<td>Amplification</td>
<td>Amplification of <strong>MYC</strong></td>
</tr>
<tr>
<td>PD3644</td>
<td>Amplification</td>
<td>Amplification of <strong>MYC</strong></td>
</tr>
<tr>
<td>PD3828</td>
<td>Amplification</td>
<td>Amplification of <strong>MYC</strong></td>
</tr>
<tr>
<td>PD3827</td>
<td>Amplification</td>
<td>Amplification of <strong>CCNE1</strong></td>
</tr>
<tr>
<td>PD3640</td>
<td>Amplification</td>
<td>Amplification of <strong>GATA6</strong></td>
</tr>
<tr>
<td>PD3642</td>
<td>Amplification</td>
<td>Amplification of <strong>KRAS</strong> (G12V mutated allele)</td>
</tr>
</tbody>
</table>