Drugging the R-loop interactome: RNA-DNA hybrid binding proteins as targets for cancer therapy⋆,⋆⋆

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ABSTRACT

Unravelling the origin of genetic alterations from point mutations to chromosomal rearrangements was greatly enhanced by the discovery of RNA-DNA hybrids (R-loops) that behave as hotspots of genomic instability in a variety of organisms. Current models suggest that uncontrolled R-loops are a hazard to genome integrity, therefore, identifying proteins that are involved in recognising and signalling R-loop structures are of key importance. Herein we analysed key RNA-DNA hybrid binding proteins in humans taking advantage of large-scale gene expression, survival rate, and drug-sensitivity data from cancer genomics databases. We show that expression of RNA-DNA hybrid binding proteins in various cancer types is associated with survival and may have contrasting outcomes in responding to therapeutic treatments. Based on the revealed pharmacogenomic landscape of human RNA-DNA hybrid binding proteins, we propose that R-loops and R-loop binding proteins are potentially relevant new epigenetic markers and therapeutic targets in multiple cancers.

1. Introduction

R-loops are special, three-stranded nucleic acid structures, composed of an RNA-DNA hybrid and a non-template, single-stranded DNA. R-loops have been implicated in a number of human diseases including repeat-expansion disorders, neurological syndromes, and cancer [1–3]. The molecular symptoms of cancer resemble the genome instability phenotype of human cell lines that accumulate R-loops and undergo replication/transcriptional stress-induced DNA damage [4]. Oncogenic mutation of HRAS^{V12}, for instance, has been shown to increase the protein levels of endogenous RNaseH1 (the enzyme that specifically degrades RNA-DNA hybrids) [5], which further supports a mechanistic link between R-loop formation and tumorigenesis.

It is well-established that single-agent therapy seldom leads to cure in oncology, therefore identifying novel targets for combination therapies is necessary [6–8]. Unfortunately, drug-targeting is not feasible in the case of tumour suppressor gene inactivating (loss-of-function) mutations (constituting most driver mutations in cancer [6], therefore, finding alternative targets could help prevent the development of drug resistance to targeted therapies. Since R-loops are universal by-products of transcription [9–11], the R-loop tolerance of cancer cells could be exploited to sensitize certain tumours to chemotherapeutic treatments. Reactivating the endogenous response of cancers to R-loop structures and R-loop-mediated genetic damage (R-loop intolerance) may make tumours more vulnerable to DNA damaging agents and cell death, which might potentially lead to the development of new combination therapies involving cytotoxic regimens and R-loop-targeting drugs. Various ligands are able target DNA-RNA hybrids, including i) small molecule intercalators that directly react with RNA-DNA hybrids, e.g. ethidium bromide, the aminoglycosides
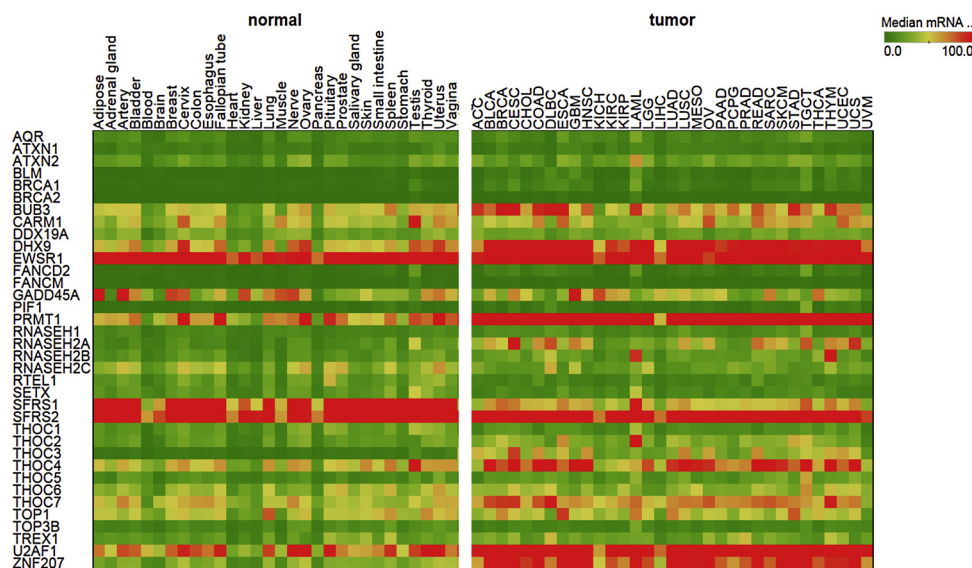

Fig. 1. Median mRNA expression levels of R-loop genes in healthy tissues and in primary tumours, extracted from the Cancer Genome Atlas database. The intensity of green and red colours is proportional to expression levels.

neomycin and paramomycin, actinomycin D [12,13], ii) G quadruplex (G4) ligands since G4s form in a similar genomic context to R-loops [14,15], e.g. Quarfloxin [16] (CX-3543; targeting rDNA G4s; phase II clinical trial completed for treatment of low grade neuroendocrine carcinoma - NCT00780663), BSU1051 [15] (targeting human telomeric G4s), TMPyP4 [17] (targeting G4s in the c-Myc promoter), pyridostatin [18] (PDS; targeting telomeric G4s and proto-oncogene tyrosine-protein kinase Src), Braco-19, perylenes and naphthalene diimides, bisquinolinium derivatives (Phen-DC(3/6) [19], iii) drugs targeting RNA-DNA hybrid-binding proteins, which have been identified recently by high-throughput screening [20,21], e.g. TDRL-505 analogues targeting RPA [22], or YK-4-279 targeting EWS-FLI1 [23].

There is evidence that R-loops are targetable by anticancer drugs to revert pathological phenotype [24,25]. For instance, in synovial and Ewing sarcoma (SS/ES), the use of clinical ATR inhibitors (ATRi) led to the accumulation of R-loops and increased sensitivity to chemotherapy [26,27]. PARP inhibitors augmented the antitumor activity of ATRi of SS cells, suggesting that combination therapies using ATRi are promising new approaches to treat sarcomas [26]. The anti-tumour drugs trabectedin and lurbinectedin have been shown to induce replicative stress and cell death in an R-loop dependent manner [28]. Using a cell line system where R-loops were stabilized by depleting THOC1 or BRCA2, cells with increased R-loop levels were more sensitive to trabectedin treatment. Consistently, cancers that accumulate R-loops in the absence of BRCA1/2 or Fanconi anaemia proteins show higher sensitivity to trabectedin therapy [29,30].

The EWS-FLI fusion protein has been shown to increase R-loop formation and inactivates BRCA1 that makes Ewing sarcoma cell lines hypersensitive to genotoxic drugs such as etoposide, camptothecin, and PARP inhibitors [31]. Since mutations in EWSR1 and its homologues are associated with several therapeutically challenging cancers, clastogenic agents that augment R-loop mediated stress could be administered as potentially effective co-therapeutic treatments in various tumours that are associated with EWSR1 mutations.

Specific G4 ligands (PDS, Braco-19, and FG) induce R-loop-mediated DNA damage and cell death in human cancer cells [18], establishing a link between the toxic effects of G4 ligands and R-loop formation. CX-5461 is another G4 ligand showing specific toxicity against BRCA-deficient cancer cells and patient-derived xenografts [16]. Repair of DNA damage induced by CX-5461 required BRCA and NHEJ repair pathways. CX-5461 is now in an advanced phase I clinical trial for patients with BRCA1/2 deficient tumours (NCT02719977).

EZM2302 is a selective inhibitor of the histone arginine methylase CARM1, an enzyme that affects R-loop homeostasis by recruiting TOP3B to RNA-DNA hybrid structures [32]. EZM2302 exhibits anti-proliferative effects both in vitro and in vivo [33]. GSK3368715 is a specific inhibitor of the PRMT1 histone arginine methyltransferase that has also been linked to R-loop metabolism [32] and being developed for the treatment of diffuse large B cell lymphoma and solid tumours (phase I clinical trial; NCT03666988).

Several other compounds have also been described to increase R-loop levels, including topoisomerase 1 inhibitors [34] (promoting R-loops by modulating the superhelicity of DNA), spliceosome [35–38] inhibitors (promoting R-loops through the retention of intronic sequences), and reactive aldehydes [39]. Furthermore, the RNA-DNA degrading enzyme RNase H2 has been recognised as a putative anti-cancer drug target [40]. With the recently identified RNase H2 inhibitors [41], RNaseH2 might serve as an effective cancer target to stabilize RNA-DNA hybrids. Finally, a series of anticancer sulphonamides have been shown to induce proteasomal degradation of the U2AF-related splicing factor coactivator of activating protein-1 in human cancer cell lines [37,42], designating targeted protein degradation by E3 ubiquitin ligases as a potent drug target for selective inactivation of splicing factors to increase R-loop levels for cancer therapy.

In the current study we aimed to investigate whether key R-loop-binding proteins are associated with cancer survival and drug sensitivity. We performed a systematic pharmacogenomic analysis to identify these associations that may suggest that R-loop formation processes in cancer cells could be exploited as biomarkers, therapeutic targets, as well as be used to sensitize certain tumours to chemotherapeutic treatments. Our results offer new avenues for epigenetic therapies that are based on modifying R-loop levels in tumours.

2. Materials and methods

2.1. Tumour types included in the analysis

We included in our analysis 33 primary cancer types available from the Cancer Genome Atlas (TCGA) [43]. These were: Adrenocortical carcinoma (ACC), Bladder urothelial carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical and endocervical cancers (CESC), Cholangiocarcinoma (CHOL), Colon adenocarcinoma (COAD), Lymphoid Neoplasm Diffuse Large B-cell Lymphoma (DLBC), Esophageal
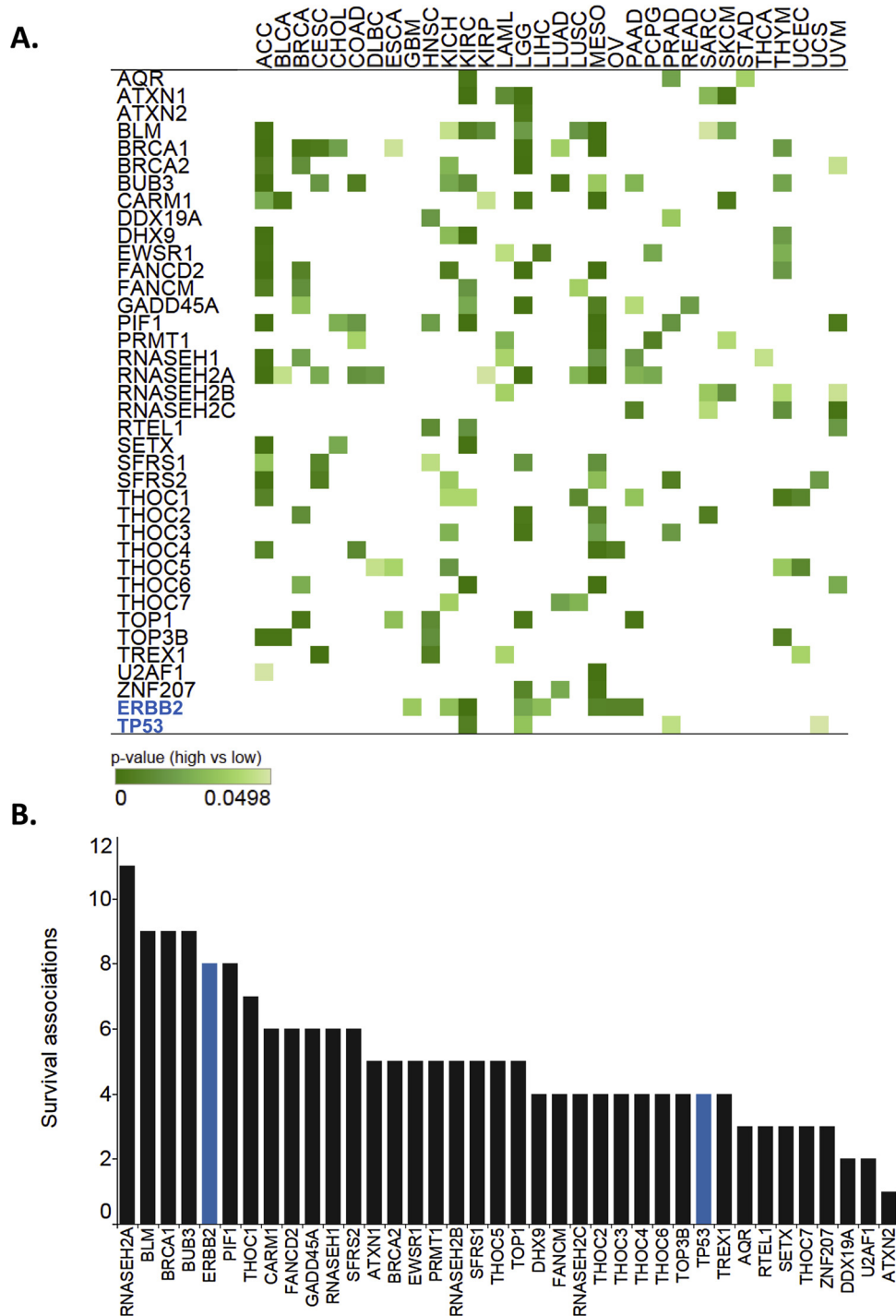
Fig. 2. Association of R-loop gene expression and survival rate in various cancer types. **(A)** Association heatmap showing the correlation of R-loop gene expression and overall survival (OS) in TCGA tumours. Statistical significance ($p < 0.05$) is highlighted in green. The number of significant associations is 179. **(B)** Histogram showing the distribution of survival associations across the studied R-loop genes. The ERBB2 (HER2) oncogene and the TP53 tumour suppressor gene are known positive controls regarding cancer association (highlighted in blue), having no established R-loop function.

carcinoma (ESCA), Glioblastoma multiforme (GBM), Head and Neck squamous cell carcinoma (HNSC), Kidney Chromophobe (KICH), Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Acute Myeloid Leukaemia (LAML), Brain Lower Grade Glioma (LGG), Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), Mesothelioma (MESO), Ovarian serous cystadenocarcinoma (OV), Pancreatic adenocarcinoma (PAAD), Pheochromocytoma and Paraganglioma (PCPG), Prostate adenocarcinoma (PRAD), Rectum adenocarcinoma (READ), Sarcoma (SARC), Skin Cutaneous Melanoma (SKCM), Stomach adenocarcinoma (STAD), Testicular Germ Cell Tumours (TGCT), Thyroid carcinoma (THCA), Thymoma (THYM), Uterine Corpus Endometrial Carcinoma (UCEC), Uterine Carcinosarcoma (UCS), and Uveal Melanoma (UVM).

2.2. Gene expression and survival data analysis

Gene expression data (RNA-sequencing) from the tumour samples

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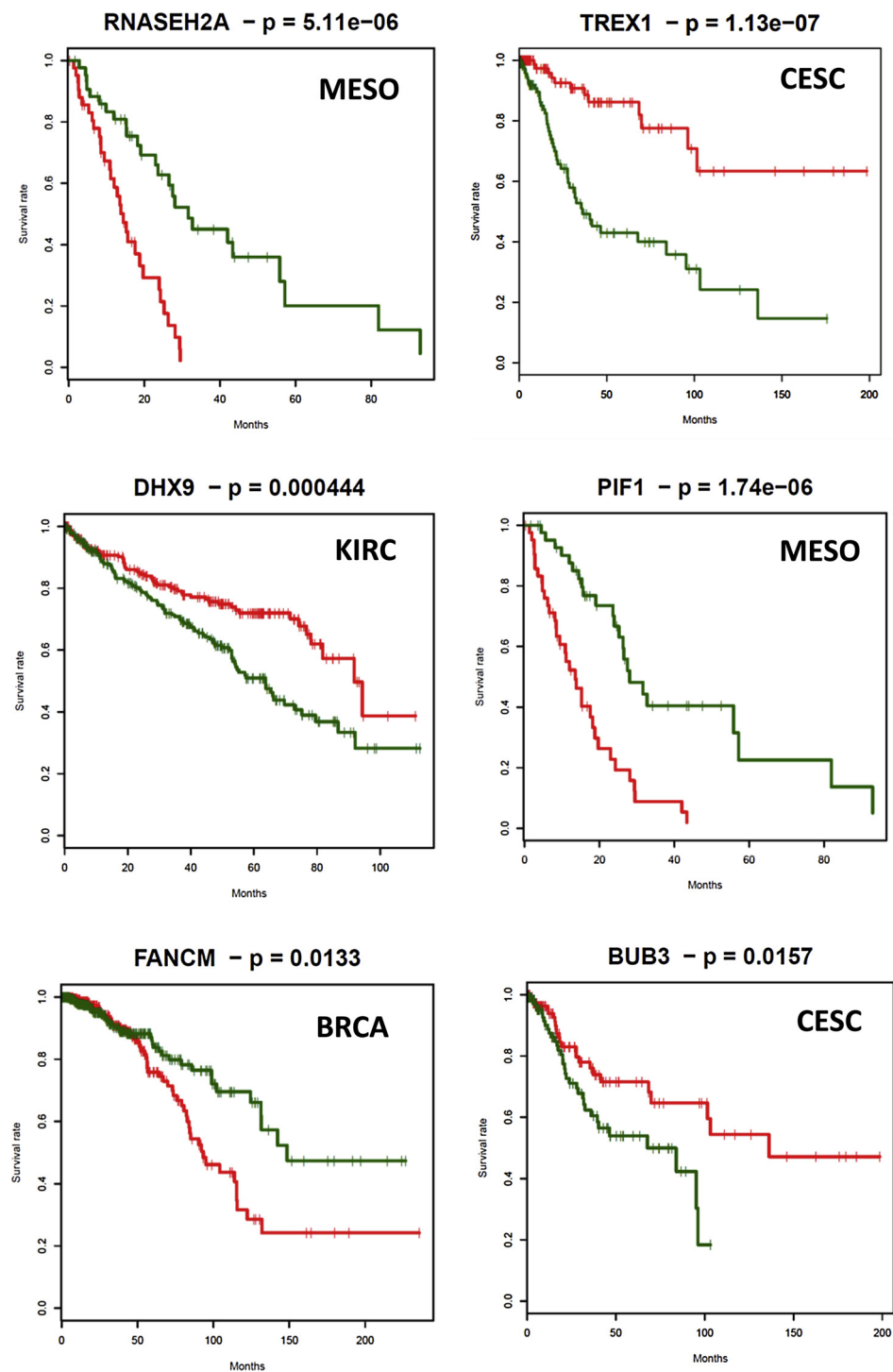
Fig. 3. Representative survival plots showing the overall survival (OS) of high expressor (red) and low expressor (green) groups in various cancer types. R-loop genes, cancer IDs, and p-values are indicated. The p-values from Kaplan-Meier analyses of all R-loop genes can be found in Table S1_3.

and overall survival time data were downloaded from TCGA project [43,44]. Healthy tissue gene expressions were obtained from the website of the Genotype-Tissue Expression (GTEx) project [45]. Survival analysis was performed and Kaplan-Meier plots were made using the "survival" package of the R software. The level of significance was p < 0.05. All p-values and adjusted p-values - corrected for multiple testing using the Benjamini-Hochberg method - are included in Table S1.

2.3. Cell line analyses

IC50 values of cancer cell lines upon treatment with 276 anticancer drugs were obtained from the Genomics of Drug Sensitivity in Cancer (GDSC) database as described and analysed previously [46]. We also downloaded additional cell line and drug information from the web page of the GDSC project [47]. Drug sensitivity and gene expression associations of cancer cell lines were presented as the Spearman correlation of IC50 values and mRNA expression scores of the R-loop genes analysed. We considered both negative and positive correlations with p-
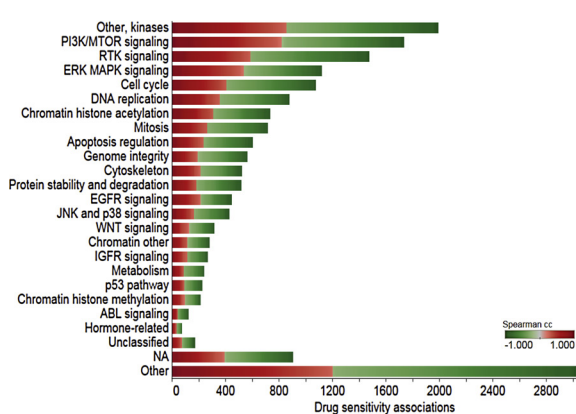
Fig. 4. Drug sensitivity associations of R-loop genes grouped by molecular pathways and drug targets. *Left:* Distribution of drug interactions over molecular pathways. Green and red colours indicate higher or lower drug sensitivity, respectively, associated with the mRNA expression levels of R-loop genes. The level of significance is p < 0.05. The number of significant associations is 22,414. *Right:* Statistical representation of drug targets showing significant drug sensitivity interactions with the R-loop genes analysed. Circle sizes are proportional to drug target frequencies.

values < 0.05. All p-values and adjusted p-values using Benjamini-Hochberg correction are included in Table S1.

2.4. RNA-DNA hybrid binding proteins

RNA-DNA hybrid binding proteins of various categories having clear R-loop function were selected for detailed analyses: AQR [48,49], ATXN1/2 [50–52], BLM [53], BRCA1/2 [54,55], BUB3 [36], BUGZ (ZNF207) [36], CARM1 [32], DDX19A [56], DHX9 [21,35], EWSR [31], FANCD2 [57,58], FANCM [58,59], GADD45A [60], PIF1 [61,62], PRMT1 [32], RNASEH1 [63,64], RNASEH2 [65,66], RTEL1 [17], SETX [48,67,68], SRSF1 [69], SRSF2 [37], THO/TREX [70], TOP1 [71–74], TOP3B [32], U2AF1 [37].

3. Results and discussion

To examine the relationship between R-loop genes and cancer, we first determined how many of the recently identified RNA-DNA hybrid binding proteins [20] are represented in the registry of cancer genes [75] representing the largest repository of 2372 genes whose somatic mutations have cancer driver roles. Of the 448 R-loop genes examined, 92 were cancer genes (**Table S1_1**) showing statistically significant enrichment (p = 0.005792; two prop z test). Next, we selected a core set of R-loop genes for a detailed analysis of tumorigenesis (n = 36), representing prominent molecular pathways with clear R-loop association that are also implicated in the formation of cancer (e.g. DNA topology; RNA-DNA hybrid ribonucleases and helicases; RNA processing, splicing and export; DNA damage; chromatin modifications; **Table S1_2**). The mRNA expression of these R-loop genes was extracted from the Cancer Genome Atlas (TCGA) database, allowing us to identify differences in the gene expression signatures of healthy tissues and primary tumours (Fig. 1). For instance, expression of BUB3, DHX9, PRMT1, THOC4, THOC7, U2AF1 and ZNF207 (BUGZ) was increased in several primary tumours compared to normal tissues, while SRSF1 (ASF/SF2) was downregulated in most cancers (except for acute myeloid leukaemia; LAML). Next, we asked whether R-loop gene expression levels correlate with survival rate of cancer patients. Taking advantage of gene expression (RNA-seq) and overall survival (OS) data from the TCGA, we generated 12,862 Kaplan-Meier survival curves in our analysis and identified numerous cases showing significant survival association with R-loop gene expression levels (179 at p < 0.05; 33 at FDR < 0.05; Fig. 2, **Supplementary** Fig. S1, and **Table S1_3**). In 70% of cases (123 at p < 0.05), low expression of R-loop genes was found to be associated with prolonged survival of cancer patients. Low expression of RNASEH2A, THOC6, PRMT1, and PIF1, for instance, significantly lengthened survival time in mesothelioma (MESO), while low FANCM mRNA level was advantageous for breast cancer survival (BRCA; (representative survival plots of low- and high-expressor groups are shown in Fig. 3). In 30% of cases (52 at p < 0.05), high expression

of R-loop genes was detected and associated with better survival; for example, TREX1 and BUB3 were beneficial for cervical squamous cell carcinoma and endocervical adenocarcinoma survival (CESC; Fig. 3 and **Table S1_3**). In the case of 10 R-loop genes, long-term survival was observed exclusively in the low-expressor group of patients, irrespective of cancer type (ATXN2 (1), BRCA2 (5), CARM1 (6), DDX19A (2), RNASEH1 (6), THOC2 (4), THOC3 (4), TOP1 (5), U2AF1 (2), ZNF207 (3). For the remaining 26 R-loop genes, high or low expressing groups varied by cancer type (**Supplementary** Fig. S2). Most survival associations were observed for adrenocortical carcinoma (19 genes) and mesothelioma (18 genes) (Fig. 2B) correlated with low expression of R-loop genes (**Table S1_3**). According to the number of survival associations (Fig. 2B), RNASEH2A, BLM, BRCA1, BUB3, and PIF1 appears to be generally important for survival in multiple cancers, while the effects of ATXN2, DDX19A and U2AF1 are limited to specific types of cancer.

To show that our approach can identify real relationships, we included an oncogene (ERBB2 - HER2) and a tumour suppressor (TP53) in our analysis that are known positive controls for cancer association, with no established R-loop function (Fig. 2, highlighted in blue). R-loop gene expressions were associated with survival in a similar number of cancers (or more in a few cases) as ERBB2 and TP53, which demonstrates the relevance of our observations on RNA-DNA hybrid binding proteins.

In order to identify R-loop genes that might serve as potential drug targets for therapeutic intervention, we sought to find synergistic interactions between the mRNA expression status of R-loop genes in a large collection of cancer cell lines and sensitivity to chemotherapeutics approved by the US Food and Drug Administration (FDA). Pharmacogenomic (RNA-seq and drug sensitivity) data for 267 anticancer compounds across 1065 cancer cell lines were extracted from the GDSC database [47], covering a wide range of biological pathways including protein kinase signalling, cytoskeleton, DNA replication, DNA repair, and cell cycle control. The total number of significant interactions showed considerable variations in the number of drug-specific associations (22,414 at p < 0.05; 508 at FDR < 0.05; range: 70–230 interactions per drug; **Supplementary** Fig. S3 and **Table S1_4**) and in the molecular pathways/drug targets involved (Fig. 4). Highly represented pathways included the Ser/Thr protein kinase pathway and PI3K/RTK/MAPK signalling (Fig. 4, **left panel**), while the most common (top10) drug targets were MEK1/2, BRAF, PARP1/2, HSP90, AKT1/3, GSKs, PI3Ks, IGF1R, ROCK1/3, and EGFR (Fig. 4, **right panel**). We found significant drug sensitivity associations in 80% of the studied R-loop genes (29 at p < 0.05; except for BRCA2, BUGZ, DDX19, RNASEH1, RTEL1, THOC3, and THOC4; **Table S1_4**), of which CARM1, EWSR1, DHX9, and THOC1 showed the highest number of drug interactions (Fig. 5; **Table S1_4**). This highlights the importance of considering R-loop gene mRNA expression levels as these may affect drug response. However, we observed significant variability in the number of drug interactions between various cancer cells lines. For
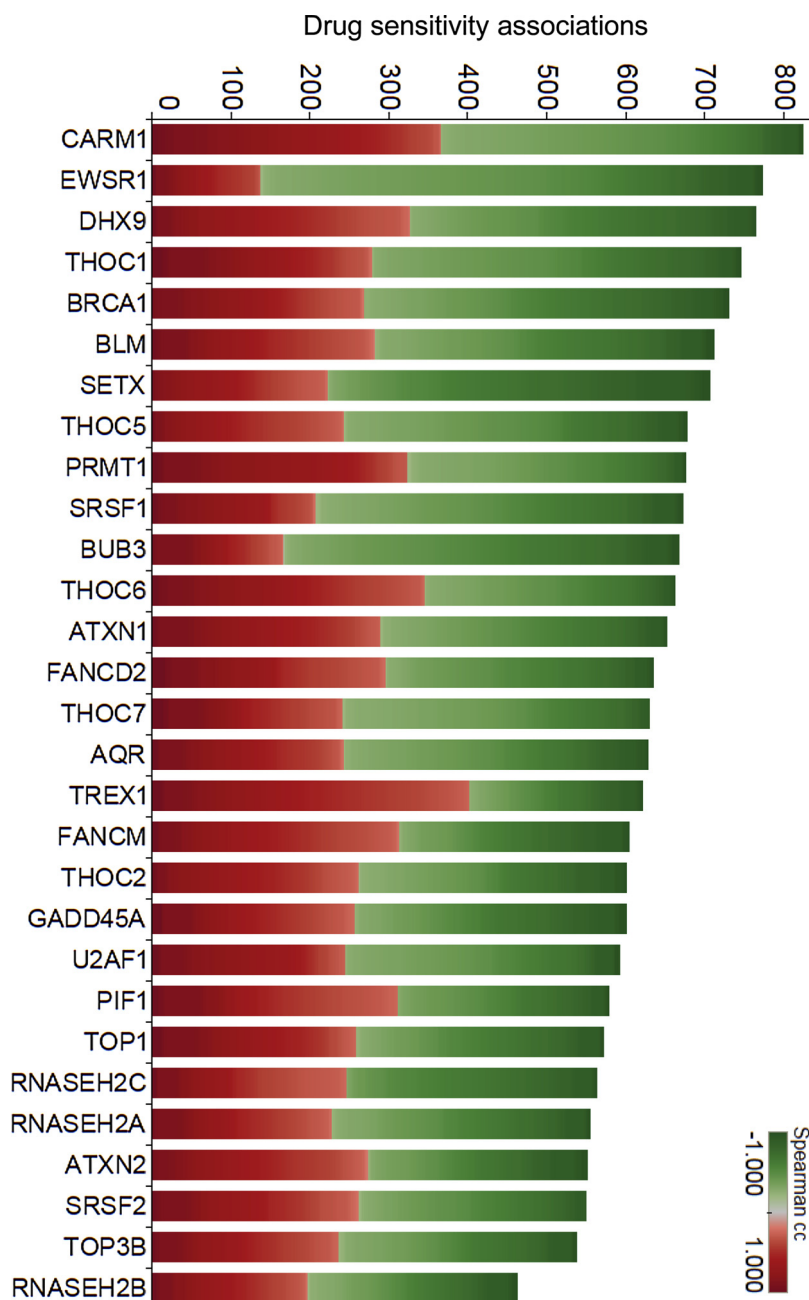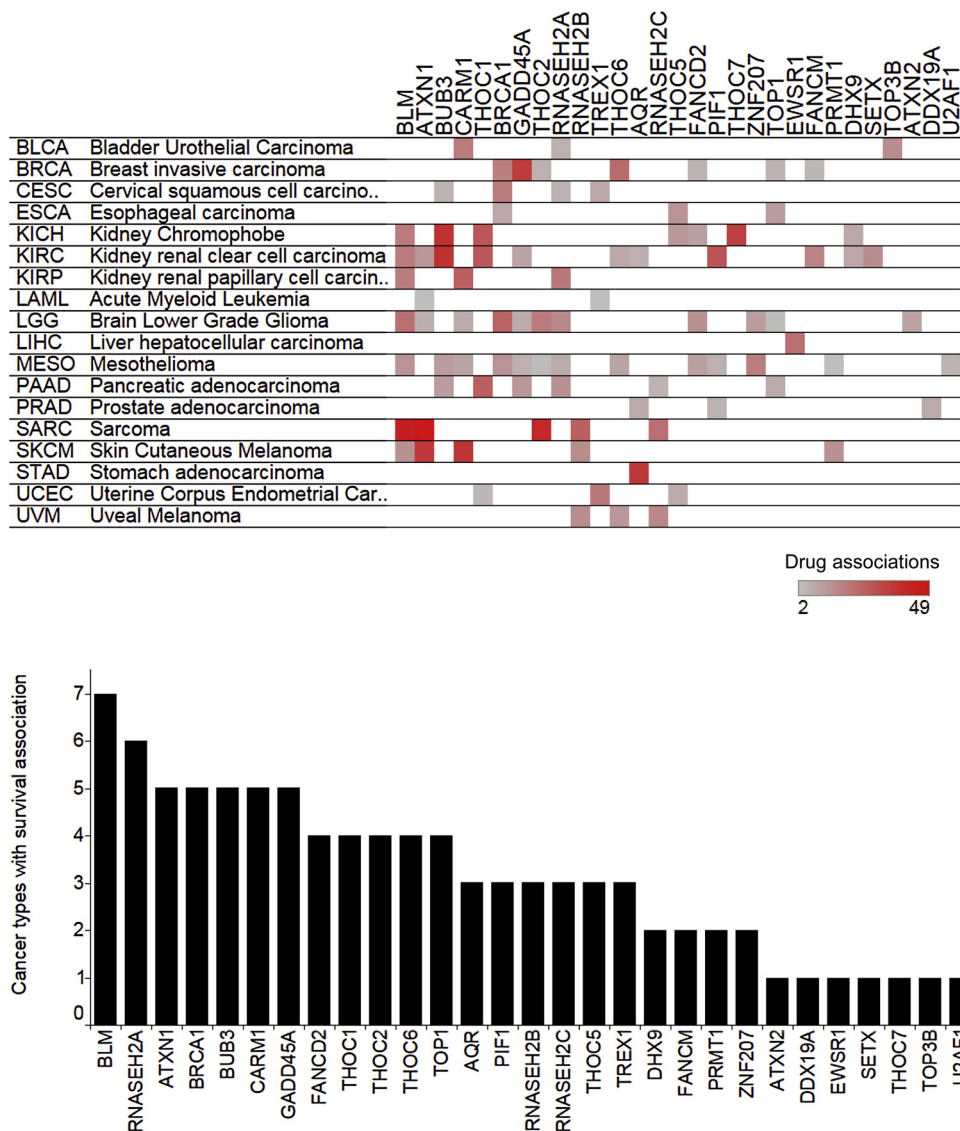
Fig. 5. Drug sensitivity associations of R-loop genes in various cancer cell lines. Green and red colours show significant negative/positive correlations between the expression of R-loop genes and drug sensitivities, indicating higher drug sensitivity or resistance of tumour cells against cancer therapeutic drugs. The level of significance is $p < 0.05$. The number of significant associations is 22,414.

example, lung small cell carcinoma and ovarian cancer cells were sensitive to most of the compounds tested, but B-cell leukaemia, Hodgkin's lymphoma, head and neck cancer, and Ewing sarcoma cells exhibited significantly lower drug efficacy (**Supplementary** Fig. S4, **green bars**). These differences indicate that RNA-DNA hybrid binding proteins may have contrasting outcomes in responding to various chemotherapeutic treatments depending on genomic context/cancer type. For example, in many cancer cell lines, high expression of TREX was associated with high IC_{50} values (half maximal inhibitory concentration) for most drug treatments indicating worse efficacy (i.e. higher drug concentration achieving half-maximal response) when TREX is overexpressed. This is entirely consistent with a recent study demonstrating that reducing the level of TREX1 leads to improved sensitivity of glioma and melanoma cells to the anticancer drugs topotecan, nimustine, and fotemustine [76]. Chondrosarcoma, lymphoblastic T cell

leukaemia, biliary tract cancer, breast cancer, and pancreatic cancer cell lines showed exceptionally high IC_{50} values (resistance) to drug treatments in the case of increased BUB3 expression. In contrast, significant negative correlations were observed between BUB3, EWSR1, and SETX expression levels and IC_{50} values in most (but not all) cancer cell lines, i.e., overexpression of these R-loop genes typically made cancer cells more sensitive to drug treatment. These results collectively indicate that targeting TREX, BUB3, SETX, and EWSR to reduce or increase their expression levels in the above tumours may help increase the efficacy of cancer chemotherapeutics.

Since cancer cell lines - deriving from natural tumours - recapitulate the genomic context and tissue type of primary cancers [47,77], we narrowed down the identified drug interactions to the fraction of R-loop genes that showed significant survival associations in the matching primary cancer (drug and survival data in non-matching cell types were

Fig. 6. Survival associations of R-loop genes in primary cancers that show significant drug sensitivity associations in the matching cancer cell line. The colour of survival association map (upper panel) highlights the number of drug interactions. Histogram (lower panel) shows the distribution of survival associations across the studied R-loop genes with significant drug interactions. The level of significance is p < 0.05. The number of significant associations is 1630.

excluded from further analysis; Fig. 6). Collectively, we identified 1,630 significant survival & drug associations (at p < 0.05) related to the expression of 29 R-loop genes (**Table S1_4**; seven R-loop genes were omitted because there was no survival or drug association, or the primary tumour could not be parsed with the corresponding tumour cell line). We observed the following trends:

i) most survival interactions (top10) were related to BLM, RNASEH2A, ATXN1, BRCA1, BUB3, CARM1, GADD45A, FANCD2, THOC1, and THOC2 associated with various cancers (Fig. 6).
ii) high expression of THOC2 in sarcoma cell lines was associated with a high IC$_{50}$ value to CX-5461 (G4 ligand/RNA polymerase I inhibitor), suggesting that CX-5461 may be less effective for the treatment of sarcomas showing high THOC2 expression. On the other hand, high expression of PIF1, FANCD2 and BRCA1 was associated with low IC$_{50}$ (sensitivity) to CX-5461 and low survival in mesothelioma (**Table S1_4**). Somewhat differently, low IC$_{50}$ to CX-5461 was associated with high expression of ATXN1 and TREX1 and increased survival in melanoma and endometrial cancer, respectively (**Table S1_4**).
iii) BRCA1 expression in oesophageal carcinoma cell lines were

associated with high IC$_{50}$ (resistance) to 5-fluorouracil (5-FU), while low BRCA1 level was associated with better survival in oesophageal carcinoma. It follows that 5-FU treatment may be efficient for the therapy of BRCA1(-) oesophageal carcinomas. Similarly, BRCA1 expression in glioma cell lines was associated with reduced efficacy to (52)-7-oxozeaenol, GDC0941, refametimib, and selumetinib, while low BRCA1 level was beneficial for the survival of brain lower grade glioma. Moreover, mesothelioma cell lines are resistant to doxorubicin, or OSU-03012 or thapsigargin when expressing BRCA1. These drugs are potentially effective chemotherapeutics for BRCA(-) gliomas and mesotheliomas. Importantly, 5-FU is being tested in a clinical trial related to oesophageal carcinoma (NCT00052910), selumetinib is being investigated in low-grade glioma (NCT01089101), and doxorubicin is evaluated in patients with mesothelioma (NCT00634205).
iv) Most drug/gene associations were observed for RDEA119, selumetinib, and olaparib with most cell lines showing resistance to RDEA119 and selumetinib, and sensitivity to olaparib associated showing high R-loop gene expression levels (**Supplementary** Fig. S3). For instance, Selumetinib treatment was ineffective in stomach adenocarcinoma cell lines overexpressing AQR (NCT02448290 -

Phase 2), in sarcoma cell lines overexpressing ATXN1 or BLM (NCT03155620 - Phase 2, NCT01752569 - Phase 2), in glioma cell lines overexpressing BRCA1 or RNASEH2A, in kidney cancer cell lines overexpressing FANCM or RNASEH2A, and in endometrial carcinoma cell lines overexpressing TREX1. In terms of patient survival, low expression of AQR, ATXN1, BLM, BRCA1 and RNASEH2A was associated with longer survival in the matching primary cancers, except for FANCM and TREX1 where higher mRNA level was beneficial for kidney renal clear cell carcinoma and endometrial carcinoma patients, respectively. Olaparib treatment was effective in prostate adenocarcinoma cells showing high DDX19A expression, and in the breast cancer cells overexpressing GADD45A, but mesothelioma cell lines with high ZNF207 (BUGZ) expression turned out to be resistant to the treatment. Olaparib is in clinical trial for patients with mesothelioma (NCT03531840 - Phase 2), prostate adenocarcinoma (NCT01682772 - Phase 2) and breast cancer (NCT02000622 - Phase 3).

v) Several drugs that are now in clinical studies to treat breast cancer show low IC_{50} values in breast cancer cell lines with high BRCA1 gene expression. For example, rucaparib (NCT02505048 - Phase 2, BRCA1,2 mutant patients), dabrafenib (NCT02465060 - Phase 2, BRAF V600E/R/K/D mutant patients), vismodegib (NCT02465060 - Phase 2 - SMO or PTCH1 mutant patients; NCT02694224 - Phase 2), BMS-754807 (NCT01225172 - Phase 2), and ruxolitimib (NCT01562873 - Phase 2, NCT02928978 - Phase 2).

vi) Oesophageal carcinoma cell lines with high BRCA1 gene expression were associated with low IC_{50} values to motesamib, cisplatin and trametinib treatments, which are now being tested in various clinical trials (NCT00101907 - Phase 1, NCT00655876 - Phase 3, NCT02465060 - Phase 2).

vii) In glioma cell lines, low CARM1 expression was associated with better efficacy to SN38 treatment (type I topoisomerase inhibitor, active metabolite of irinotecan), while glioma patients with low CARM1 levels showed longer survival. It is possible that CARM1(-) cancers are more susceptible to SN38 drug treatment leading to inhibition of Top3B and Top1, which could in turn increase R-loop levels [32] and induce cell death.

The above pharmacogenomic associations of human RNA-DNA hybrid binding proteins support the role of R-loop genes in tumorigenesis and in determining the efficiency of tumour therapies. We note that, however, our analytical approach represents a hypothesis generating exercise performed on multi-experiment observations to identify all possible associations of R-loop genes and human malignancies, which should be experimentally validated in further high-content analysis projects. Our data suggest that modulating the expression levels of R-loop genes may affect clinical responses to anticancer drug treatments, and these expression changes could be used to define patient groups who are most likely to benefit from a therapy. Based on the revealed pharmacogenomic interactions, we propose that R-loops and R-loop binding proteins are potentially relevant new epigenetic markers and therapeutic targets in multiple cancers. Further exploration of the recognised associations is expected to improve drug effectiveness and identify potential combination therapeutics.

Funding

Appendix A. Supplementary data

References

[1] M.P. Crossley, M. Bocek, K.A. Cimprich, R-loops as cellular regulators and genomic threats, Mol. Cell 73 (2019) 398–411, https://doi.org/10.1016/j.molcel.2019.01.024.

[2] P. Richard, J.L. Manley, R loops and links to human disease, J. Mol. Biol. (2016), https://doi.org/10.1016/j.jmb.2016.08.031.

[3] K. Skourti-Stathaki, N.J. Proudfoot, A double-edged sword: R loops as threats to genome integrity and powerful regulators of gene expression, Genes Dev. 28 (2014) 1384–1396, https://doi.org/10.1101/gad.242990.114.Freely.

[4] A. Aguilera, T. García-Muse, R loops: from transcription byproducts to threats to genome stability, Mol. Cell 46 (2012) 115–124, https://doi.org/10.1016/j.molcel.2012.04.009.

[5] P. Kotsantis, L.M. Silva, S. Irmscher, R.M. Jones, L. Folkes, N. Gromak, E. Petermann, Increased global transcription activity as a mechanism of replication stress in cancer, Nat. Commun. 7 (2016) 13087, https://doi.org/10.1038/ncomms13087.

[6] B. Vogelstein, N. Papadopoulos, V.E. Velculescu, S. Zhou, L.A. Diaz Jr., K.W. Kinzler, Cancer Genome Landscapes, Science 339 (80-) (2013) 1546–1558, https://doi.org/10.1126/science.1235122.

[7] R.M. Webster, Combination therapies in oncology, Nat. Rev. Drug Discov. 15 (2016) 81–82, https://doi.org/10.1038/nrd.2016.3.

[8] M. Gianni, Y. Qin, G. Wenes, B. Bandstra, A.P. Conley, V. Subbiah, R. Leibowitz-Amit, S. Ekmekcioglu, E.A. Grimm, J. Roszik, High-throughput architecture for discovering combination Cancer therapeutics, JCO Clin Cancer Inform. 2 (2018) 1–12, https://doi.org/10.1200/CCI.17.00054.

[9] L.A. Sanz, S.R. Hartono, Y.W. Lim, P.A. Ginno, L.A. Sanz, S.R. Hartono, Y.W. Lim, S. Steyaert, A. Rajpurkar, P.A. Ginno, X. Xu, F. Chédin, Prevalent, Dynamic, and Conserved R-Loop Structures Associate with Specific Epigenomic Signatures in Mammals, Mol. Cell 63 (2016) 167–178, https://doi.org/10.1016/j.molcel.2016.05.032.

[10] L. Halász, Z. Karányi, B. Boros-oláh, T. Kuik-rózsa, É. Sipos, É. Nagy, Á. Mosolygó-l, A. Mázló, É. Rajnavölgyi, G. Halmos, L. Székvölgyi, RNA-DNA hybrid (R-loop) immunoprecipitation mapping: an analytical workflow to evaluate inherent biases, Genome Res. 27 (2017) 1063–1073, https://doi.org/10.1101/gr.219394.

[11] S. Hamperl, K.A. Cimprich, The contribution of co-transcriptional RNA: DNA hybrid structures to DNA damage and genome instability, DNA Repair (Amst). 19 (2014) 84–94, https://doi.org/10.1016/j.dnarep.2014.03.023.

[12] N.N. Shaw, D.P. Arya, Recognition of the unique structure of DNA:RNA hybrids, Biochimie. 90 (2008) 1026–1039, https://doi.org/10.1016/j.biochi.2008.04.011.

[13] F. Takusagawa, K.T. Takusagawa, R.G. Carlson, R.F. Weaver, Selectivity of F8-Actinomycin D for RNA:DNA hybrids and its anti-leukemia activity, Bioorg. Med. Chem. Lett. 5 (1997) 1197–1207, https://doi.org/10.1016/S0968-0896(97)00062-X.

[14] S.Q. Mao, A.T. Ghanbarian, J. Spiegel, S. Martínez Cuesta, D. Beraldi, M. Di Antonio, G. Marsico, R. Hänsel-Hertsch, D. Tannahill, S. Balasubramanian, DNA G-quadruplex structures mold the DNA methylome, Nat. Struct. Mol. Biol. 25 (2018) 951–957, https://doi.org/10.1038/s41594-018-0131-8.

[15] A.J. Angelbello, J.L. Chen, J.L. Childs-Disney, P. Zhang, Z.F. Wang, M.D. Disney, Using genome sequence to enable the design of medicines and chemical probes, Chem. Rev. 118 (2018) 1599–1663, https://doi.org/10.1021/acs.chemrev.7b00504.

[16] H. Xu, M. Di Antonio, S. McKinney, V. Mathew, B. Ho, N.J. O'Neil, N. Dos Santos, J. Silvester, V. Wei, J. Garcia, F. Kabeer, D. Lai, P. Soriano, J. Banáth, D.S. Chiu, D. Yap, D.D. Le, F.B. Ye, A. Zhang, K. Thu, J. Soong, S.C. Lin, A.H.C. Tsai, T. Osako, T. Algara, D.N. Saunders, J. Wong, J. Xian, M.B. Bally, J.D. Brenton, G.W. Brown, S.P. Shah, D. Cescon, T.W. Mak, C. Caldas, P.C. Stirling, P. Hieter, S. Balasubramanian, S. Aparicio, CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours, Nat. Commun. 8 (2017), https://doi.org/10.1038/ncomms14432.

[17] J.B. Vannier, V. Pavicic-Kaltenbrunner, M.I.R. Petalcorin, H. Ding, S.J. Boulton, RTEL1 dismantles T loops and counteracts telomeric G4-DNA to maintain telomere integrity, Cell. 149 (2012) 795–806, https://doi.org/10.1016/j.cell.2012.03.030.

[18] A. De Magis, S.G. Manzo, M. Russo, J. Marinello, O. Sordet, R. Morigi Capranico, DNA damage and genome instability by G-quadruplex ligands are mediated by R-loops in human cancer cells, Proc. Natl. Acad. Sci. 116 (2018) 816–825, https://doi.org/10.1073/pnas.1810409116.

[19] E. Ruggiero, S.N. Richter, G-quadruplexes and G-quadruplex ligands: targets and tools in antiviral therapy, Nucleic Acids Res. 46 (2018) 3270–3283, https://doi.org/10.1093/nar/gky187.

[20] I.X. Wang, C. Grunseich, J. Fox, J. Burdick, Z. Zhu, N. Ravazian, M. Hafner, V.G. Cheung, Human proteins that interact with RNA / DNA hybrids, Genome Res. (2018) 1–10, https://doi.org/10.1101/gr.237362.118.

[21] A. Cristini, M. Groh, M.S. Kristiansen, N. Gromak, RNA/DNA hybrid interactome identifies DXH9 as a molecular player in transcriptional termination and R-Loop-Associated DNA damage, Cell Rep. 23 (2018) 1891–1905, https://doi.org/10.1016/j.celrep.2018.04.025.

[22] S.C. Shuck, J.J. Turchi, Targeted inhibition of replication protein A reveals cytotoxic activity, synergy with chemotherapeutic DNA-damaging agents, and insight

into cellular function, Cancer Res. 70 (2010) 3789–3798, https://doi.org/10.1158/0008-5472.CAN-09-3422.

[23] T.Z. Minas, J. Han, T. Javaheri, S.-H. Hong, M. Schlederer, Y. Saygideğer-Kont, H. Çelik, K.M. Mueller, I. Temel, M. Özdemirli, H. Kovar, H.V. Erkizan, J. Toretsky, L. Kenner, R. Moriggl, A. Üren, YK-4-279 effectively antagonizes EWS-FLI1 induced leukemia in a transgenic mouse model, Oncotarget. 6 (2015) 37678–37694, https://doi.org/10.18632/oncotarget.5520.

[24] D. Colak, N. Zaninovic, M.S. Cohen, Z. Rosenwaks, W. Yang, Promoter-bound tri-nucleotide repeat mRNA drives epigenetic silencing in fragile X syndrome, Science (80) (2014) 1002–1005, https://doi.org/10.1126/science.1245831.

[25] W.T. Powell, R.L. Coulson, M.L. Gonzales, F.K. Crary, S.S. Wong, S. Adams, R. a Ach, P. Tsang, N.A. Yamada, D.H. Yasui, F. Chédin, J.M. LaSalle, R-loop formation at Snord116 mediates topotecan inhibition of Ube3a-antisense and allele-specific chromatin decondensation, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 13938–13943, https://doi.org/10.1073/pnas.1305426110.

[26] S.E. Jones, E.D.G. Fleuren, J. Frankum, A. Konde, C.T. Williamson, D.B. Krastev, H.N. Pemberton, J. Campbell, A. Gulati, R. Elliott, M. Menon, J.L. Selfe, R. Brough, S.J. Pettitt, W. Niedzwiedz, W.T.A. Van Der Graaf, J. Shipley, A. Ashworth, C.J. Lord, ATR is a therapeutic target in synovial sarcoma, Cancer Res. 77 (2017) 7014–7026, https://doi.org/10.1158/0008-5472.CAN-17-2056.

[27] M. Nieto-Soler, I. Morgado-Palacin, V. Lafarga, E. Lecona, M. Murga, E. Callen, D. Azorin, J. Alonso, A.J. Lopez-Contreras, A. Nussenzweig, O. Fernandez-Capetillo, Efficacy of ATR inhibitors as single agents in Ewing sarcoma, Oncotarget. 7 (2016) 58759–58767, https://doi.org/10.18632/oncotarget.11643.

[28] E. Tumini, E. Herrera-Moyano, M. San Martín-Alonso, S. Barroso, C.M. Galmarini, A. Aguilera, The antitumor drugs trabectedin and lurbinectedin induce transcription-dependent replication stress and genome instability, Mol. Cancer Res. (2018), https://doi.org/10.1158/1541-7786.MCR-18-0575 molcanres.0575.2018.

[29] J.A. Casado, P. Rio, E. Marco, V. Garcia-Hernandez, A. Domingo, L. Perez, J.C. Tercero, J.J. Vaquero, B. Albella, F. Gago, J.A. Bueren, Relevance of the Fanconi anemia pathway in the response of human cells to trabectedin, Mol. Cancer Ther. 7 (2008) 1309–1318, https://doi.org/10.1158/1535-7163.MCT-07-2432.

[30] B.J. Monk, D. Lorusso, A. Italiano, S.B. Kaye, M. Aracil, A. Tanović, M. D'Incalci, Trabectedin as a chemotherapy option for patients with BRCA deficiency, Cancer treat. Rev. 50 (2016) 175–182, https://doi.org/10.1016/j.ctrv.2016.09.009.

[31] A. Gorthi, J.C. Romero, E. Loranc, L. Cao, L.A. Lawrence, E. Goodale, A.B. Iniguez, X. Bernard, V.P. Masamsetti, S. Roston, E.R. Lawlor, J.A. Toretsky, K. Stegmaier, S.L. Lessnick, Y. Chen, A.J.R. Bishop, EWS-FLI1 increases transcription to cause R-Loops and block BRCA1 repair in Ewing sarcoma, Nature. 555 (2018) 387–391, https://doi.org/10.1038/nature25748.

[32] Y. Yang, K.M. McBride, S. Hensley, Y. Lu, F. Chedin, M.T. Bedford, Arginine me-thylation facilitates the recruitment of TOP3B to chromatin to prevent r loop ac-cumulation, Mol. Cell 53 (2014) 484–497, https://doi.org/10.1016/j.molcel.2014.01.011.

[33] A.E. Drew, O. Moradei, S.L. Jacques, N. Rioux, A.P. Boriack-Sjodin, C. Allain, M.P. Scott, L. Jin, A. Raimondi, J.L. Handler, H.M. Ott, R.G. Kruger, M.T. McCabe, C. Sneeringer, T. Riera, G. Shapiro, N.J. Waters, L.H. Mitchell, K.W. Duncan, M.P. Moyer, R.A. Copeland, J. Smith, R. Chesworth, S.A. Ribich, Identification of a CARM1 inhibitor with potent in vitro and in vivo activity in preclinical models of multiple myeloma, Sci. Rep. 7 (2017) 17993, https://doi.org/10.1038/s41598-017-18446-z.

[34] J. Marinello, S. Bertoncini, I. Aloisi, A. Cristini, G.M. Tagliazucchi, M. Forcato, O. Sordet, G. Capranico, Dynamic effects of topoisomerase i inhibition on R-loops and short transcripts at active promoters, PLoS One 11 (2016) 1–18, https://doi.org/10.1371/journal.pone.0147053.

[35] P. Chakraborty, J.T.J. Huang, K. Hiom, DHX9 helicase promotes R-loop formation in cells with impaired RNA splicing, Nat. Commun. 9 (2018) 4346, https://doi.org/10.1038/s41467-018-06677-1.

[36] Y. Wan, X. Zheng, H. Chen, Y. Guo, H. Jiang, X. He, X. Zhu, Y. Zheng, Splicing function of mitotic regulators links R-loop-mediated DNA damage to tumor cell killing, J. Cell Biol. 209 (2015) 235–246, https://doi.org/10.1083/jcb.201409073.

[37] L. Chen, J.Y. Chen, Y.J. Huang, Y. Gu, J. Qiu, H. Qian, C. Shao, X. Zhang, J. Hu, H. Li, S. He, Y. Zhou, O. Abdel-Wahab, D.E. Zhang, X.D. Fu, The augmented R-Loop is a unifying mechanism for myelodysplastic syndromes induced by high-risk spli-cing factor mutations, Mol. Cell 69 (2018) 412–425, https://doi.org/10.1016/j.molcel.2017.12.029 e6.

[38] S. Sorrells, S. Nik, M. Casey, R.C. Cameron, H. Truong, C. Toruno, M. Gulfo, A. Lowe, C. Jette, R.A. Stewart, T.V. Bowman, Spliceosomal components protect embryonic neurons from R-loop-mediated DNA damage and apoptosis, Dis. Model. Mech. 11 (2018), https://doi.org/10.1242/dmm.031583 pii: dmm031583.

[39] S.L.W. Tan, S. Chadha, Y. Liu, E. Gabasova, D. Perera, K. Ahmed, S. Constantinou, X. Renaudin, M. Lee, R. Aebersold, A.R. Venkitaraman, A class of environmental and endogenous toxins induces BRCA2 haploinsufficiency and genome instability, Cell. 169 (2017) 1105–1118, https://doi.org/10.1016/j.cell.2017.05.010 e15.

[40] J.M. Flanagan, J.M. Funes, S. Henderson, L. Wild, N. Carey, C. Boshoff, Genomics screen in transformed stem cells reveals RNASEH2A, PPAP2C, and ADARB1 as putative anticancer drug targets, Mol. Cancer Ther. 8 (2009) 249–260, https://doi.org/10.1158/1535-7163.MCT-08-0636.

[41] R. White, B. Saxty, J. Large, C.A. Kettleborough, A.P. Jackson, Identification of small-molecule inhibitors of the ribonuclease H2 enzyme, J. Biomol. Screen. 18 (2013) 610–620, https://doi.org/10.1177/1087057113476550.

[42] T. Uehara, Y. Minoshima, K. Sagane, N.H. Sugi, K.O. Mitsuhashi, N. Yamamoto, H. Kamiyama, K. Takahashi, K. Kotake, M. Uesugi, A. Yokoi, A. Inoue, T. Yoshida, M. Mabuchi, A. Tanaka, T. Owa, Selective degradation of splicing factor CAPERα by anticancer sulfonamides, Nat. Chem. Biol. 13 (2017) 675–680, https://doi.org/10.1038/nchembio.2363.

[43] The Cancer genome atlas research network, J.N. Weinstein, Eric A. Collisson, Gordon B. Mills, Kenna R.Mills Shaw, Chris Sander, K. Ellrott, Joshua M. Stuart, B.A. Ozenberger, I. Shmulevich, The cancer genome atlas pan-cancer analysis project, Nat. Genet. 45 (2013) 1113–1120, https://doi.org/10.1038/ng.2764.

[44] R.L. Grossman, A. Heath, M. Murphy, Toward data science as a service, Comput. Sci. Eng. 18 (2016) 10–20, https://doi.org/10.1109/MCSE.2016.92.

[45] B.E. Stranger, L.E. Brigham, R. Hasz, M. Hunter, C. Johns, M. Johnson, G. Kopen, W.F. Leinweber, J.T. Lonsdale, A. McDonald, B. Mestichelli, K. Myer, B. Roe, M. Salvatore, S. Shad, J.A. Thomas, G. Walters, M. Washington, J. Wheeler, J. Bridge, B.A. Foster, B.M. Gillard, E. Karasik, R. Kumar, M. Miklos, M.T. Moser, S.D. Jewell, R.G. Montroy, D.C. Rohrer, D.R. Valley, D.A. Davis, D.C. Mash, S.E. Gould, P. Guan, S. Koester, A.R. Little, C. Martin, H.M. Moore, A. Rao, J.P. Struewing, S. Volpi, K.D. Hansen, P.F. Hickey, L.F. Rizzardi, L. Hou, Y. Liu, B. Molinie, Y. Park, N. Rinaldi, L. Wang, N. Wittenberghe, M. Claussnitzer, E.T. Gelfand, Q. Li, S. Linder, R. Zhang, K.S. Smith, E.K. Tsang, L.S. Chen, K. Demanelis, J.A. Doherty, J. Farzana, M.G. Kibriya, L. Jiang, S. Lin, M. Wang, R. Jian, X. Li, J. Chan, D. Bates, M. Diegel, J. Halow, E. Haugen, A. Johnson, R. Kaul, K. Lee, M.T. Maurano, J. Nelson, F.J. Neri, R. Sandstrom, M.S. Fernando, C. Linke, M. Oliva, A. Skol, F. Wu, J.M. Akey, A.P. Feinberg, J.L. Billy, B.L. Pierce, J.A. Stamatoyannopoulos, H. Tang, K.G. Ardlie, M. Kellis, M.P. Snyder, S.B. Montgomery, Enhancing GTEx by bridging the gaps between genotype, gene expression, and disease, Nat. Genet. 49 (2017) 1664–1670, https://doi.org/10.1038/ng.3969.

[46] Y. Qin, A.P. Conley, E.A. Grimm, J. Roszik, A tool for discovering drug sensitivity and gene expression associations in cancer cells, PLoS One 12 (2017) 1–6, https://doi.org/10.1371/journal.pone.0176763.

[47] W. Yang, J. Soares, P. Greninger, E.J. Edelman, H. Lightfoot, S. Forbes, N. Bindal, D. Beare, J.A. Smith, I.R. Thompson, S. Ramaswamy, P.A. Futreal, D.A. Haber, M.R. Stratton, C. Benes, U. McDermott, M.J. Garnett, Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells, Nucleic Acids Res. 41 (2013) 955–961, https://doi.org/10.1093/nar/gks1111.

[48] J. Sollier, C.T. Stork, M.L. García-Rubio, R.D. Paulsen, A. Aguilera, K.A. Cimprich, Transcription-coupled nucleotide excision repair factors promote R-Loop-Induced genome instability, Mol. Cell 56 (2014) 777–785, https://doi.org/10.1016/j.molcel.2014.10.020.

[49] R. Sakasai, M. Isono, M. Wakasugi, M. Hashimoto, Y. Sunatani, T. Matsui, A. Shibata, T. Matsunaga, K. Iwabuchi, Aquarius is required for proper CtIP ex-pression and homologous recombination repair, Sci. Rep. 7 (2017) 13808, https://doi.org/10.1038/s41598-017-13695-4.

[50] L.A. Ostrowski, A.C. Hall, K. Mekhail, Ataxin-2: from RNA control to human health and disease, Genes (Basel). 8 (2017) 2–21, https://doi.org/10.3390/genes8060157.

[51] J.S. Salvi, J.N.Y. Chan, K. Szafranski, T.T. Liu, J.D. Wu, J.B. Olsen, N. Khanam, B.P.K. Poon, A. Emili, K. Mekhail, Roles for Pbp1 and caloric restriction in genome and lifespan maintenance via suppression of RNA-DNA hybrids, Dev. Cell 30 (2014) 177–191, https://doi.org/10.1016/j.devcel.2014.05.013.

[52] K.J. Abraham, J.N.Y. Chan, J.S. Salvi, B. Ho, A. Hall, E. Vidya, R. Guo, S.A. Killackey, N. Liu, J.E. Lee, G.W. Brown, K. Mekhail, Intersection of calorie restriction and magnesium in the suppression of genome-destabilizing RNA–DNA hybrids, Nucleic Acids Res. 44 (2016), https://doi.org/10.1093/nar/gkw752 gkw752.

[53] E. Yun, C. Chang, C.A. Novoa, M.J. Aristizabal, Y. Coulombe, R. Segovia, R. Chaturvedi, Y. Shen, C. Keong, A.S. Tam, S.J.M. Jones, J.Y. Masson, M.S. Kobor, P.C. Stirling, RECQ-like helicases Sgs1 and BLM regulate R-loop-associated genome instability, J. Cell Biol. 216 (2017) 3991–4005, https://doi.org/10.1083/jcb.201703168.

[54] E. Hatchi, K. Skourti-Stathaki, S. Ventz, L. Pinello, A. Yen, K. Kamieniarz-Gdula, S. Dimitrov, S. Pathania, K.M. McKinney, M.L. Eaton, M. Kellis, S.J. Hill, G. Parmigiani, N.J. Proudfoot, D.M. Livingston, BRCA1 recruitment to transcrip-tional pause sites is required for R-loop-driven DNA damage repair, Mol. Cell 57 (2015) 636–647, https://doi.org/10.1016/j.molcel.2015.01.011.

[55] V. Bhatia, S.I. Barroso, M.L. García-Rubio, E. Tumini, E. Herrera-Moyano, A. Aguilera, BRCA2 prevents R-loop accumulation and associates with TREX-2 mRNA export factor PCID2, Nature. 511 (2014) 362–365, https://doi.org/10.1038/nature13374.

[56] D. Hodroj, B. Recolin, K. Serhal, S. Martinez, N. Tsanov, R. Abou Merhi, D. Maiorano, An ATR-dependent function for the Ddx19 RNA helicase in nuclear R-loop metabolism, EMBO J. 36 (2017) 1182–1198, https://doi.org/10.15252/embj.201695131.

[57] M.L. García-Rubio, C. Pérez-Calero, S.I. Barroso, E. Tumini, E. Herrera-Moyano, I.V. Rosado, A. Aguilera, The fanconi Anemia pathway protects genome integrity from R-loops, PLoS Genet. 11 (2015) 1–17, https://doi.org/10.1371/journal.pgen.1005674.

[58] Z. Liang, F. Liang, Y. Teng, X. Chen, J. Liu, S. Longerich, T. Rao, A.M. Green, N.B. Collins, Y. Xiong, L. Lan, P. Sung, G.M. Kupfer, Binding of FANCI-FANCD2 complex to RNA and R-Loops stimulates robust FANCD2 monoubiquitination, Cell Rep. 26 (2019) 564–572, https://doi.org/10.1016/J.CELREP.2018.12.084 e5.

[59] R.A. Schwab, J. Nieminuszczy, F. Shah, J. Langton, D. Lopez Martinez, C.C. Liang, M.A. Cohn, R.J. Gibbons, A.J. Deans, W. Niedzwiedz, The fanconi Anemia pathway maintains genome stability by coordinating replication and transcription, Mol. Cell 60 (2015) 351–361, https://doi.org/10.1016/j.molcel.2015.09.012.

[60] K. Arab, E. Karaulanov, M. Musheev, P. Trnka, A. Schäfer, I. Grummt, C. Niehrs, GADD45A binds R-loops and recruits TET1 to CpG island promoters, Nat. Genet. (2019), https://doi.org/10.1038/s41588-018-0306-6.

[61] P.L.T. Tran, T.J. Pohl, C.-F. Chen, A. Chan, S. Pott, V.A. Zakian, PIF1 family DNA helicases suppress R-loop mediated genome instability at tRNA genes, Nat. Commun. 8 (2017) 15025, https://doi.org/10.1038/ncomms15025.

[62] J.-B. Boulé, V. a Zakian, The yeast Pif1p DNA helicase preferentially unwinds RNA DNA substrates, Nucleic Acids Res. 35 (2007) 5809–5818, https://doi.org/10.1093/nar/gkm613.

[63] a Arudchandran, S. Cerritelli, S. Narimatsu, M. Itaya, D.Y. Shin, Y. Shimada, R.J. Crouch, The absence of ribonuclease H1 or H2 alters the sensitivity of Saccharomyces cerevisiae to hydroxyurea, caffeine and ethyl methanesulphonate: implications for roles of RNases H in DNA replication and repair, Genes Cells 5 (2000) 789–802.

[64] R.W. Maul, H. Chon, K. Sakhuja, S.M. Cerritelli, L.A. Gugliotti, P.J. Gearhart, R.J. Crouch, R-loop depletion by over-expressed RNase H1 in mouse B cells increases activation-induced deaminase access to the transcribed strand without altering frequency of isotype switching, J. Mol. Biol. (2017) 1–9, https://doi.org/10.1016/j.jmb.2016.12.020.

[65] R. Uehara, S.M. Cerritelli, N. Hasin, K. Sakhuja, M. London, J. Iranzo, H. Chon, A. Grinberg, R.J. Crouch, Two RNase H2 mutants with differential rNMP processing activity reveal a threshold of ribonucleotide tolerance for embryonic development, Cell Rep. 25 (2018) 1135–1145, https://doi.org/10.1016/j.celrep.2018.10.019 e5.

[66] K.J. Mackenzie, P. Carroll, L. Lettice, A. Revuelta, E. Abbondati, R.E. Rigby, B. Rabe, F. Kilanowski, G. Grimes, A. Fluteau, P.S. Devenney, R.E. Hill, M.A.M. Reijns, A.P. Jackson, Ribonuclease H2 mutations induce a cGAS / STING-dependent innate immune response, EMBO J. (2016) 1–14, https://doi.org/10.15252/embj.201593339.

[67] O.J. Becherel, A.J. Yeo, A. Stellati, E.Y.H. Heng, J. Luff, A.M. Suraweera, R. Woods, J. Fleming, D. Carrie, K. McKinney, X. Xu, C. Deng, M.F. Lavin, Senataxin plays an essential role with DNA damage response proteins in meiotic recombination and gene silencing, PLoS Genet. 9 (2013), https://doi.org/10.1371/journal.pgen.1003435.

[68] M. Groh, L.O. Albulescu, A. Cristini, N. Gromak, Senataxin: genome guardian at the interface of transcription and neurodegeneration, J. Mol. Biol. 429 (2017) 3181–3195, https://doi.org/10.1016/j.jmb.2016.10.021.

[69] X. Li, J.L. Manley, Inactivation of the SR protein splicing factor ASF/SF2 results in genomic instability, Cell. 122 (2005) 365–378, https://doi.org/10.1016/j.cell.2005.06.008.

[70] B. Gómez-González, M. García-Rubio, R. Bermejo, H. Gaillard, K. Shirahige, A. Marín, M. Foiani, A. Aguilera, Genome-wide function of THO/TREX in active genes prevents R-loop-dependent replication obstacles, EMBO J. 30 (2011) 3106–3119, https://doi.org/10.1038/emboj.2011.206.

[71] W. Shen, H. Sun, C.L. De Hoyos, J.K. Bailey, X.-H. Liang, S.T. Crooke, Dynamic nucleoplasmic and nucleolar localization of mammalian RNase H1 in response to RNAP I transcriptional R-loops, Nucleic Acids Res. (2017) 1–21, https://doi.org/10.1093/nar/gkx710.

[72] A. El Hage, S. Webb, A. Kerr, D. Tollervey, Genome-wide distribution of RNA-DNA hybrids identifies RNase H targets in tRNA genes, retrotransposons and mitochondria, PLoS Genet. 10 (2014), https://doi.org/10.1371/journal.pgen.1004716.

[73] É. Hegedüs, E. Kókai, P. Nánási, L. Imre, L. Halász, R. Jossé, Z. Antunovics, M.R. Webb, A. El Hage, Y. Pommier, L. Székvölgyi, V. Dombrádi, G. Szabó, Endogenous single-strand DNA breaks at RNA polymerase II promoters in Saccharomyces cerevisiae, Nucleic Acids Res. 46 (2018) 10649–10668, https://doi.org/10.1093/nar/gky743.

[74] L. Székvölgyi, Z. Rákosy, B.L. Bálint, E. Kókai, L. Imre, G. Vereb, Z. Bacsó, K. Goda, S. Varga, M. Balázs, V. Dombrádi, L. Nagy, G. Szabó, Ribonucleoprotein-masked nicks at 50-kbp intervals in the eukaryotic genomic DNA, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 14964–14969, https://doi.org/10.1073/pnas.0702269104.

[75] S.K. Venkata, D. Repana, J. Nulsen, L. Dressler, M. Bortolomeazzi, A. Tourna, A. Yakovleva, T. Palmieri, F.D. Ciccarelli, The Network of Cancer genes (NCG): a comprehensive catalogue of known and candidate cancer genes from cancer sequencing screens, Genome Biol. 20 (2019) 1–12, https://doi.org/10.1186/s13059-018-1612-0.

[76] M.T. Tomicic, D. Aasland, T. Nikolova, B. Kaina, M. Christmann, Human three prime exonuclease TREX1 is induced by genotoxic stress and involved in protection of glioma and melanoma cells to anticancer drugs, Biochim. Biophys. Acta - Mol. Cell Res. 1833 (2013) 1832–1843, https://doi.org/10.1016/j.bbamcr.2013.03.029.

[77] J. Barretina, G. Caponigro, N. Stransky, K. Venkatesan, A.A. Margolin, S. Kim, C.J. Wilson, J. Lehár, G.V. Kryukov, D. Sonkin, A. Reddy, M. Liu, L. Murray, M.F. Berger, J.E. Monahan, P. Morais, J. Meltzer, A. Korejwa, J. Jané-Valbuena, F.A. Mapa, J. Thibault, E. Bric-Furlong, P. Raman, A. Shipway, I.H. Engels, J. Cheng, G.K. Yu, J. Yu, P. Aspesi, M. De Silva, K. Jagtap, M.D. Jones, L. Wang, C. Hatton, E. Palescandolo, S. Gupta, S. Mahan, C. Sougnez, R.C. Onofrio, T. Liefeld, L. MacConaill, W. Winckler, M. Reich, N. Li, J.P. Mesirov, S.B. Gabriel, G. Getz, K. Ardlie, V. Chan, V.E. Myer, B.L. Weber, J. Porter, M. Warmuth, P. Finan, J.L. Harris, M. Meyerson, T.R. Golub, M.P. Morrissey, W.R. Sellers, R. Schlegel, L.A. Garraway, The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity, Nature 483 (2012) 603–607, https://doi.org/10.1038/nature11003.