Supplementary Materials For

Synthetic lethality in ATM-deficient RAD50-mutant tumors underlie outlier response to cancer therapy

Hikmat Al-Ahmadie, Gopa Iyer, Marcel Hohl, Saurabh Asthana, Akiko Inagaki, Nikolaus Schultz, Aphrothiti J. Hanrahan, Sasinya N. Scott, A. Rose Brannon, Gregory C. McDermott, Mono Pirun, Irina Ostrovnaya, Philip Kim, Nicholas D. Socci, Agnes Viale, Gary K. Schwartz, Victor Reuter, Bernard H. Bochner, Jonathan E. Rosenberg, Dean F. Bajorin, Michael F. Berger, John H.J. Petrini, David B. Solit, Barry S. Taylor

Supplementary Methods

Targeted capture and sequencing

We utilized a solution phase hybridization-based exon capture and massively parallel DNA sequencing assay (IMPACT) to profile mutations and copy number alterations in 281 key cancer-associated genes in both the pre- and post-etoposide/cisplatin tumors of the index patient. Custom oligonucleotides (Nimblegen SeqCap) were designed to capture all protein-coding exons and select introns of 279 commonly implicated oncogenes, tumor suppressor genes, and members of pathways deemed actionable by targeted therapies. Capture probes targeting all exons of two additional genes of interest that were found to be mutated in the index whole genome (*RAD50* and *ATR*) were synthesized as biotinylated oligonucleotides and spiked into the hybridization reaction (Integrated DNA Technologies, xGen lockdown probes). The identities of the 281 target genes and the 119 probe sequences for *RAD50* and *ATR* are provided as in Supplementary Tables 3 and 4.

Barcoded sequence libraries were prepared according to the manufacturer's instructions (New England Biolabs) using DNA isolated from FFPE tissue. Between 199 and 250ng of genomic FFPE DNA was input for library construction. Libraries were amplified using Kapa HiFi DNA polymerase (Kapa Biosystems) for 10 cycles and quantified by Qubit (Invitrogen). Barcoded libraries were mixed at equimolar concentrations (100ng per sample) with up to 21 libraries per pool. Each pool was input to a single exon capture reaction as previously described(1). IDT capture probes for *RAD50* and *ATR* were pooled, and 10 picomoles of the oligonucleotide pool were spiked into each Nimblegen hybridization reaction. To prevent off-target hybridization, we also spiked in a separate pool of blocker oligonucleotides complementary to the full sequences of all barcoded adaptors to a final total concentration of 10 micromolar. DNA was subsequently sequenced on an Illumina HiSeq 2000 to generate paired-end 75-bp reads.

Sequence analysis

The alignment, processing, and variant detection analyses (point mutations, insertions and deletions, copy number alterations, and structural rearrangements) were performed for the tumor and matched normal whole genome sequences all as previously described (2). Due to the phenotype-to-genotype nature of this WGS analysis, we assessed somatic nonsynonymous mutations, CNAs, and rearrangements in the context of the observed phenotype, durable treatment response. We evaluated the membership of genes bearing candidate mutations in the proximal pathways up- and down-stream of the mechanism of action of both AZD7762 and irinotecan. Additionally, germline SNPs were identified in the matched normal genome using the

UnifiedGenotyper and 94 genes previously implicated in drug metabolism (drug transporters, metabolizing enzymes, and related genes) were screened for the presence of variants that may contribute to the clinical response to therapy. None were observed after excluding common polymorphisms. Mutation rates were calculated from only those sequenced bases that were covered at sufficient depth and quality for variant calling (88.6% of total), which was performed with Mutect(3) and the Genome Analysis Toolkit (GATK)(4). IMPACT data was analyzed similarly. Briefly, sequence data were demultiplexed using CASAVA, adapter sequence was trimmed from the 3' ends, and reads were aligned to the reference human genome (hg19) using the Burrows-Wheeler Alignment tool v0.6.2(5). Duplicate masking, local realignment, and quality score recalibration were performed using GATK v2.3-9 according to GATK best practices(4). Local realignment was performed jointly for samples coming from the same patient. We achieved an average unique sequence coverage of 250-fold per tumor sample. IMPACT sequence data were analyzed to identify three classes of somatic alterations: single-nucleotide variants, small insertions/deletions (indels), and copy number alterations. Single-nucleotide variants were called using muTect v1.0.27783(3), and indels were called using the SomaticIndelDetector tool in GATK. All candidate mutations and indels were reviewed manually(6). The mean sequence coverage for each target region was calculated using the GATK DepthOfCoverage and was used to compute copy number as described previously(1). Increases and decreases in the coverage ratios (tumor:normal) were used to infer amplifications and deletions, respectively.

To assess the clonality of individual mutations (Supplementary Fig. 3) detected by whole-genome sequencing in the index responder, we performed analysis with the ABSOLUTE algorithm using total copy number segmentation inferred from the WGS data and a MAF file of somatic mutations to calculate purity and ploidy and estimate the fraction of cancer cells harboring each somatic mutation(7). While we sought to assess the significance of mutual exclusivity in the pattern of mutations within Mre11 complex members (*RAD50*, *MRE11A*, and *NBN*) in the cross-cancer cohort, due to the rate of such mutations (~4%), we lacked sufficient power to test the significance of this event among the 7,494 tumors available. Nevertheless, only those tumors that were hyper- or ultra-mutated, defined as having a mutation count greater than 1.5x the interquartile range of the distribution of all mutation counts by tumor type, harbored co-occurring mutations in two more genes, and when excluded, indicate mutations in Mre11 complex members are mutually exclusive. Organism abbreviations and Uniprot accession numbers for Rad50 D-loop conservation are: Hs, human (Q92878); Mm, mouse (P70388); Dm, fly (Q9W252); At, arabadopsis (Q9SL02); Sc, budding yeast (P12753); Sp, fission yeast (Q9UTJ8); Ec, E. coli (C8TIY7); Pf, P. furiosus (P58301); T4, bacteriophage T4 (P04522).

In silico analysis of the predicted functional significance of mutations

All somatic mutations identified from WGS in the index patient were assessed computationally for their effect on protein structure and function initially with Mutation Assessor(8), and then manually inspected after integration with DNA copy number alterations and structural rearrangements. Aside from TP53 A161T, the ATR H585D and RAD50 L1237F mutations were deemed likeliest to directly affect the machinery of checkpoint control and DNA repair. The criteria used to predict which of these mutations likely served as the basis of the exceptional response in this patient are described in the main text. In addition to these, the *ATR* locus (3q23), unlike *RAD50*, was not affected by a focal copy number alteration (CNA). Instead, a low-level genomic gain spanned the majority of the q-arm of chromosome 3, but the CNA appeared to be subclonal and the mutation was heterozygous. Additionally, an ATR abnormality does not appear to correlate with the observed phenotype of profound response to the combination of checkpoint inhibition and mutagenic chemotherapy. Indeed, if ATR function was impaired then

one would predict checkpoint inhibition with AZD7762 would be less effective due to the diminished ATR-dependent activation of the pathway. Additionally, *mec1Δ* mutants were ~100-fold less sensitive than *rad50Δ* mutants to the same concentration of CPT (data not shown). Together, these results indicated that mutant ATR would not be predicted to cause sensitivity to AZD7762.

MRN complex mutations in human cancer
The mutational status of *RAD50*, *MRE11A*, and *NBN*, the three genes that comprise the Mre11 complex, were assessed across cancer types in the following manner. First, the reported somatic mutations in public studies of exome or genome sequencing in cancer were converted into MAF file format (https://wiki.nci.nih.gov/display/TCGA/Mutation+Annotation+Format+(MAF)+Specification)(9-40). These were supplemented by the available mutation data from The Cancer Genome Atlas deposited at the Data Coordinating Center (DCC) for public access (http://cancergenome.nih.gov/). Non-synonymous mutations affecting any of the three genes were considered and subsequently annotated for their sequence context and affect with Oncotator (http://www.broadinstitute.org/oncotator/). For the purposes of this analysis, we excluded synonymous, intronic, and potential regulatory mutations (those appearing in the 5' or 3' untranslated regions or flanking sequence). Due to the variable reporting of sequencing data in the literature, we were unable to calculate a conventional mutation rate per million bases for each tumor and study, predominantly due to the absence of the number of unique bases sufficiently sequenced for variant calling in each of the studies and samples. We did, however, use the count of mutations detected in each sample as proxy for mutation rate.

Supplementary Note

To address the impact of *rad50*^{L1240F} on the activity of Tel1 (ATM), we generated cells that are dependent on the conserved TM pathway (Mre11 complex-Tel1 dependent). The TM pathway in yeast corresponds to the Mre11 complex-ATM pathway in human cells and is unmasked through the deletion of *Sae2* in a *mec1Δ* cell. *Sae2* deletion hyper-activates Tel1 to the extent that it can compensate for DNA damage response phenotypes associated with the loss of *Mec1* (ATR). Therefore, *Sae2* deletion in a *mec1Δ* cell will restore wildtype responses to checkpoint activation, as indicated by downstream targets such as Rad53. Consistent with this prediction, we show here that whereas *mec1Δ* cells are sensitized to CPT only after Sae2 loss, the opposite is true upon treatment with the DNA damaging agent methyl methanesulfonate (MMS), to which *mec1Δ* cells are initially highly sensitivity and Sae2 deletion rescues essentially wildtype MMS resistance via activation of Tel1 (*mec1*Δ and *mec1*Δ *sae2*Δ mutants respectively, Fig. 3B and Supplementary Fig. 7). This compensation requires a functional Mre11 complex. Therefore, any Mre11 complex mutation that blocks the ability to compensate for *mec1Δ* cannot activate Tel1.

Supplementary References

1. Wagle N, Berger MF, Davis MJ, Blumenstiel B, Defelice M, Pochanard P, et al. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. Cancer Discov. 2012;2:82-93.
2. Iyer G, Hanrahan AJ, Milowsky MI, Al-Ahmadie H, Scott SN, Janakiraman M, et al. Genome sequencing identifies a basis for everolimus sensitivity. Science. 2012;338:221.
3. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol. 2013.
4. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011;43:491-8.
5. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754-60.
6. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative genomics viewer. Nat Biotechnol. 2011;29:24-6.
7. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol. 2012;30:413-21.
8. Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: application to cancer genomics. Nucleic Acids Res. 2011.
9. The Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609-15.
10. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science. 2011;333:1154-7.
11. Banerji S, Cibulskis K, Rangel-Escareno C, Brown KK, Carter SL, Frederick AM, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature. 2012;486:405-9.
12. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet. 2012;44:685-9.
13. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012;483:603-7.
14. Barretina J, Taylor BS, Banerji S, Ramos AH, Lagos-Quintana M, Decarolis PL, et al. Subtype-specific genomic alterations define new targets for soft-tissue sarcoma therapy. Nat Genet. 2010;42:715-21.
15. Biankin AV, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature. 2012;491:399-405.
16. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008;455:1069-75.
17. Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet. 2012;44:760-4.
18. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature. 2012;487:239-43.

19. Guo G, Gui Y, Gao S, Tang A, Hu X, Huang Y, et al. Frequent mutations of genes encoding ubiquitin-mediated proteolysis pathway components in clear cell renal cell carcinoma. Nat Genet. 2012;44:17-9.
20. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, et al. A landscape of driver mutations in melanoma. Cell. 2012;150:251-63.
21. Imielinski M, Berger AH, Hammerman PS, Hernandez B, Pugh TJ, Hodis E, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell. 2012;150:1107-20.
22. Jones DT, Jager N, Kool M, Zichner T, Hutter B, Sultan M, et al. Dissecting the genomic complexity underlying medulloblastoma. Nature. 2012;488:100-5.
23. Lee RS, Stewart C, Carter SL, Ambrogio L, Cibulskis K, Sougnez C, et al. A remarkably simple genome underlies highly malignant pediatric rhabdoid cancers. J Clin Invest. 2012;122:2983-8.
24. Molenaar JJ, Koster J, Zwijnenburg DA, van Sluis P, Valentijn LJ, van der Ploeg I, et al. Sequencing of neuroblastoma identifies chromothripsis and defects in neuritogenesis genes. Nature. 2012;483:589-93.
25. Peifer M, Fernandez-Cuesta L, Sos ML, George J, Seidel D, Kasper LH, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet. 2012;44:1104-10.
26. Pugh TJ, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, et al. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. Nature. 2012;488:106-10.
27. Quesada V, Conde L, Villamor N, Ordonez GR, Jares P, Bassaganyas L, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. Nat Genet. 2012;44:47-52.
28. Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, et al. Novel mutations target distinct subgroups of medulloblastoma. Nature. 2012;488:43-8.
29. Rudin CM, Durinck S, Stawiski EW, Poirier JT, Modrusan Z, Shames DS, et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet. 2012;44:1111-6.
30. Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, Storm EE, Conboy CB, et al. Recurrent R-spondin fusions in colon cancer. Nature. 2012;488:660-4.
31. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature. 2012;486:395-9.
32. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature. 2012;486:400-4.
33. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011;333:1157-60.
34. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18:11-22.
35. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008;455:1061-8.
36. The Cancer Genome Atlas Research Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61-70.
37. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487:330-7.
38. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. Nature. 2012;489:519-25.

39. Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet. 2011;43:309-15.
40. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478:64-9.