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Inherited DNA repair variants are associated with clonal hematopoiesis and cardiovascular risk in men with metastatic prostate cancer

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Author Contributions

O.O. and Y.K. conceived and designed the study, analyzed data, and drafted the manuscript.

O.M., K.T., A.F., K.K., W.T., and L.A.J. contributed to data acquisition and analysis.

B.A.C., D.C., A.A.M., J.A.F., C.F., T.L.L., R.W., and L.W. provided critical review and intellectual input.

M.M.P. supervised the study and provided overall guidance.

All authors reviewed and approved the final manuscript.

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Data Sharing Statement : De-identified genomic and clinical data underlying this study are available upon reasonable request to the corresponding author, subject to Mayo Clinic institutional policies and data-sharing agreements.

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Myeloid clonal hematopoiesis (CH) arises from somatic mutations in myeloid genes within hematopoietic stem and progenitor cells[1,2]. These mutations confer a proliferative advantage, leading to clonal expansion, increased risk of myeloid neoplasms (MN), and reduced survival in patients with solid tumors[1,2]. While CH is most commonly associated with aging and involves mutations in *DNMT3A, TET2*, and *ASXL1* (collectively referred to as DTA mutations), it is also highly prevalent in solid tumors such as metastatic prostate cancer (mPC), with reported rates upto 57 %[3]. In mPC, secondary to genotoxic therapeutic exposures, there is a marked shift toward DNA damage response (DDR) gene mutations such as *TP53, PPM1D*, and *CHEK2*[3-5]. Importantly, in addition to CH, mPC is also characterized by frequent tumor-derived somatic alterations and pathogenic germline variants (PGVs), all of which may influence therapy selection and complicate genomic interpretation[6].

The increasing use of therapeutic modalities such as poly ADP ribose polymerase (PARP) inhibitors and radiopharmaceuticals (e.g. 177Lu-PSMA) raises concerns about further clonal selection[7]. Moreover, CH may influence disease trajectory and treatment tolerance[8,9]. As these therapies move earlier in the disease course, understanding the complex interactions between therapeutic exposures, germline predisposition, and somatic evolution becomes increasingly important for anticipating long-term hematologic and cardiovascular risks. However, despite increasing awareness of CH in prostate cancer, the extent to which inherited genetic variation contributes to CH acquisition and the effects of sequential therapies on clonal evolution remain poorly understood.

This study characterized the somatic mutational landscape of CH in men with metastatic castration-resistant prostate cancer (mCRPC) and examined the association between PGVs in DDR genes and somatic CH. Secondary objectives included evaluating major adverse cardiovascular events (MACE), cytopenias, MN, and overall survival (OS).

Following institutional review board approval, we identified participants from the Prostate Cancer Medically Optimized Genome Enhanced Therapy (PROMOTE) study (ClinicalTrials.gov Identifier: NCT01953640) (**See supplements/Figure S1**). All patients had histologically confirmed prostate adenocarcinoma and met criteria for mCRPC. Peripheral blood samples collected at enrollment were analyzed for somatic mutations using error-corrected next-generation sequencing (340 CH related genes). Concurrent whole-exome sequencing of matched tumor and blood samples identified germline alterations. Somatic variants with a variant allele fraction (VAF) ≥ 0.5% were classified as CH, consistent with prior error-corrected sequencing studies[10]. Variants with VAF ≥0.5% were included to capture low-frequency somatic clones with potential for clinical impact[4]. Outcomes of interest, determined retrospectively, included MACE-defined as a composite of acute coronary syndrome (ACS) and stroke/transient ischemic attack. Additional outcomes included persistent cytopenias, incident MN, and OS. Associations between genomic and clinical variables were analyzed using Fisher exact test and multivariable logistic regression. Survival was evaluated using the log-rank test.

The final cohort consisted of 67 men with mCRPC (median age 74.4 years, interquartile range 58 to 79). Thirty-one percent had *de novo* metastatic disease. All received androgen deprivation therapy (ADT) with or without androgen receptor pathway inhibitors (ARPIs) for a median of 2.6 years (IQR 1.3-4.6) prior to enrollment. Sixty-seven percent had received prior radiotherapy (pelvic n=35, palliative n=10), while only 3% (n=2) had received prior chemotherapy, both with docetaxel (**Table1**). None had prior PARP inhibitor, 177-lutetium-PSMA-617, or radium-223. Median intervals from the first treatment to blood sampling was 4.1 years for radiotherapy and 2.3 years for chemotherapy. At

CH testing, 52% of patients were anemic (94% Grade 1, 3% Grade 2, 2% as Grade 3), 6% had Grade 1 thrombocytopenia, and 1% had grade 3 neutropenia (**Table 1**). Somatic CH mutations with a VAF of ≥2% were identified in 30 of 67 participants (53%). Including exploratory lower-frequency clones (0.5-2%), overall CH prevalence increased to 88% (59/67), totaling 108 mutations (**Table S1**). Among CH-positive individuals, 39 % of mutations occurred in DTA genes, 16 % in DDR genes, including *PPM1D* which accounted for 11 % of all mutations. The most frequent mutations involved *TET2 (*16%), *DNMT3A* (14 %), and *PPM1D* (11 %). Median number of mutations per individual was two (range, 1-6), and the median VAF was 1.8 % (range, 0.5-50.2),(**Fig.1a**). Variants were categorized by VAF into micro-CH (VAF <2.0%, n=65), CH with low VAF (2% to <5%, n=28), intermediate (5.0% to <10.0%, n=5), and high (≥10.0%, n=10) VAF groups **(Fig.1b)**. Most mutations fell into micro-CH and CH with low VAF categories, comprising approximately 60% and 30% of variants, respectively. Two likely germline *ATM* variants and one likely tumor-derived *TP53* variant were excluded from analysis.

Likely pathogenic and PGVs were identified in 13 men (19 %), including *ATM* (6 %), *BRCA2* (6 %), and *TP53* (6 %) (**Fig.1c, Table S2**). On multivariable logistic regression, while there was no association between overall CH prevalence and PGV status, PGV carriers had significantly increased odds of harboring DDR-associated CH (odds ratio 8.03, 95% confidence interval 1.90 to 34.04, p = 0.0047) (**Fig.1d**), suggesting a germline contribution to somatic DDR mutation acquisition.

At a median follow-up of 2.2 years from CH testing, MACE was significantly more common in CH-positive individuals compared to CH-negative individuals (39 % [23/59] versus 0 % [0/8], p = 0.04) (**Fig.2a**). The proportion of MACE events did not differ between individuals with higher (VAF ≥10%) and lower (VAF <10%) CH clones. During the same period, 17 patients (25 %) developed persistent cytopenias (> three months). Among four who underwent bone marrow biopsy, Three had marrow involvement by metastatic prostate cancer, and one patient met criteria for clonal cytopenia of undetermined significance, demonstrating a somatic *CHEK2* mutation. No cases of MN were observed.

In two patients with serially collected blood samples, longitudinal sequencing demonstrated dynamic clonal behavior. Low-frequency CH clones identified at enrollment (VAF <5%) expanded substantially over time, including an *ATM*-mutated clone increasing from 3.9% to 37.7% after 8 years, with others, such as *KMT2D* and *TET2*, potentially regressing to undetectable limits (**Fig.2b**). These findings highlight the potential for even small, initially subclinical clones to evolve under therapeutic pressure.

Median OS from prostate cancer diagnosis was 8.6 years. At last follow-up, two patients remained alive. Most deaths were attributed to progressive metastatic disease. While CH status was not independently associated with OS (**Fig.2c**), baseline cytopenias correlated with poorer survival (median OS 1.2 years versus 2.5 years, p = 0.025).

In this prospective cohort of men with pretreated mCRPC, we observed a high prevalence of CH (53 %), with numerous low-frequency clones that may represent early clonal events capable of expansion under therapeutic pressure. Serial sampling in two individuals confirmed this trend, as low-frequency clones at baseline expanded after years of ongoing systemic therapy. These findings reinforce the hypothesis that even micro-CH clones may possess latent fitness advantages that become clinically relevant with cumulative genotoxic exposure[4]. Although prior studies have emphasized cardiovascular risk primarily among high-VAF clones, emerging data suggest a more complex,

potentially non-linear relationship in which smaller clones may still exert biological effects depending on context and inflammatory milieu[11].

We did not observe a significant association between the presence of PGVs and overall CH prevalence. However, individuals with PGVs had more than an eightfold increased likelihood of harboring DDR-associated CH. Although exploratory, this finding suggests that germline variation may influence the somatic mutational profile of the hematopoietic compartment, particularly in DDR pathway genes. Notably, a recent population-based analysis did not observe a comparable association, suggesting that germline–somatic interactions may be context-dependent[12].

In prostate cancer, PGVs in genes such as *BRCA2, ATM*, and *TP53* are established drivers of tumorigenesis. Our results extend this paradigm to the hematopoietic system, demonstrating that inherited DDR defects may shape somatic mutational patterns in nonmalignant tissues. We found that 19% of men in our cohort with mCRPC carried DDR PGVs and had a higher prevalence of DDR-associated CH, suggesting susceptibility to treatment-induced hematologic toxicity. DDR CH confers a selective advantage under genotoxic stress, potentially driving more aggressive clonal dynamics, particularly during therapies such as PARP inhibitors [13,14]. We observed a significantly higher incidence of MACE in CH-positive individuals, which was primarily driven by ACS, aligning with emerging data linking CH to cardiovascular risk[15]. Given the cardiometabolic side effects of ARPIs, CH may interact with these therapies to amplify cardiovascular toxicity. While CH status was not independently associated with overall mortality, baseline cytopenias remained the strongest predictor of poorer outcomes. Prospective longitudinal studies tracking CH evolution, cytopenias, and other adverse outcomes could enable earlier intervention, refined risk stratification, and more personalized treatment strategies in this high-risk population.

A key limitation of our study is the relatively small sample size, which may limit the power to detect modest associations and reduce generalizability.

In summary, this study defines the interplay between germline predisposition and somatic CH mutations in men with mPC treated primarily with ADT, ARPIs, and radiation. We report a high prevalence of subclonal CH mutations and demonstrate that PGVs in DDR genes might be associated with increased risk of DDR CH. Given the increasing use of genotoxic therapies in mPC and the emerging links between CH and adverse outcomes, future studies integrating serial genomic profiling, treatment exposure data, and functional validation will be essential to confirm these observations and define the biological and therapeutic implications of CH in mCRPC.

References

1. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488-2498.
2. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med. 2017;377(2):111-121.
3. Jensen JL, Bobek O, Chan ICC, et al. Clonal hematopoiesis and clinical outcomes in metastatic castration-resistant prostate cancer patients given androgen receptor pathway inhibitors (Alliance A031201). Clin Cancer Res. 2024;30(21):4910-4919.
4. Bolton KL, Ptashkin RN, Gao T, et al. Cancer therapy shapes the fitness landscape of clonal hematopoiesis. Nat Genet. 2020;52(11):1219-1226.
5. Hsu JI, Dayaram T, Tovy A, et al. PPM1D mutations drive clonal hematopoiesis in response to cytotoxic chemotherapy. Cell Stem Cell. 2018;23(5):700-713.
6. Magee D, Domenyuk V, Abraham J, et al. Characterization of plasma cell-free DNA variants as of tumor or clonal hematopoiesis origin in 16,812 advanced cancer patients. Clin Cancer Res. 2025;31(13):2710-2718.
7. Kusne Y, Mosalem OM, Quillen J, et al. Hematologic dysfunction and myeloid neoplasm risk in patients treated with lutetium-177 prostate specific antigen membrane therapy. Haematologica. 2025;110(4):1053-1062.
8. Takahashi K, Wang F, Kantarjian H, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. Lancet Oncol. 2017;18(1):100-111.
9. Pich O, Bernard E, Zagorulya M, et al. Tumor-infiltrating clonal hematopoiesis. N Engl J Med. 2025;392(16):1594-1608.
10. Karpova D, Huerga Encabo H, Donato E, et al. Clonal hematopoiesis landscape in frequent blood donors. Blood. 2025;145(21):2411-2423.
11. Díez-Díez M, Ramos-Neble BL, de la Barrera J, et al. Unidirectional association of clonal hematopoiesis with atherosclerosis development. Nat Med. 2024;30(10):2857-2866.
12. Marshall CH, Arafa AT, Jaeger E, et al. Germline DNA repair gene mutations and clonal hematopoiesis in 24,849 patients with BRCA-associated cancers. Cancers (Basel). 2025;17(9):1432.
13. Silver AJ, Bick AG, Savona MR. Germline risk of clonal haematopoiesis. Nat Rev Genet. 2021;22(9):603-617.
14. Liu J, Tran D, Xue L, et al. Germline genetic variation impacts clonal hematopoiesis landscape and progression to malignancy. Nat Genet. 2025;57(8):1023-1034.
15. Uddin MDM, Nguyen NQH, Yu B, et al. Clonal hematopoiesis of indeterminate potential, DNA methylation, and risk for coronary artery disease. Nat Commun. 2022;13(1):5350-5362.

Table 1

Table 1. Baseline clinical and laboratory characteristics of the cohort stratified by clonal hematopoiesis (CH) status

Characteristic	Total (n=67)	CH (n=59)	No CH (n=8)	P value
Age – median (IQR), years	74 (58–79)	75 (70–79)	73 (71–79)	0.43
History of prior chemotherapy	2 (3%)	2 (3%)	0	1.00
History of prior radiation	45 (67%)	38 (64%)	7 (88%)	0.30
Pelvic irradiation	35 (52%)	28 (47%)	7 (88%)	0.06
Gleason score – n (%)				
≤ 6	8 (12%)	6 (10%)	2 (25%)	0.20
7	20 (30%)	18 (31%)	2 (25%)	1.00
≥ 8	34 (51%)	32 (54%)	2 (25%)	0.10
Missing	5 (7%)	3 (5%)	2 (25%)	0.10
Baseline PSA – median (IQR)	11 (6–27)	11 (6–27)	11 (3–22)	0.40
Hemoglobin, g/dL – median (IQR)	12.5 (11.8–13.3)	12.5 (11.8–13.3)	12.7 (11.5–13.75)	0.95
Platelets, K/μL – median (IQR)	200 (162–230)	200 (161–233)	198 (173–219)	0.73
WBC, K/μL – median (IQR)	5.8 (5.1–7.6)	5.8 (5.2–7.8)	5.3 (4.7–6.12)	0.25
Anemia:				
Any grade (%)	35 (52)	31 (53)	4 (50)	1
Grade 1 (%)	33 (49)	29 (49)	4 (50)	
Grade 2 (%)	1 (1)	1 (2)	0	
Grade 3 (%)	1 (1)	1 (2)	0	
Thrombocytopenia:				
Any grade (%)	6 (9)	6 (10)	0	0.57
Grade 1 (%)	6 (9)	6 (10)	0	
Neutropenia:				
Any grade (%)	1 (1)	1 (1)	0	1
Grade 3 (%)	1 (1)	1 (1)		

Figure Legends:

Figure 1 panel. Somatic and germline mutational landscape in metastatic prostate cancer

A. Somatic clonal hematopoiesis landscape in metastatic prostate cancer
Oncoplot depicting all somatic clonal hematopoiesis (CH) variants identified in the cohort. Variant classes are color-coded: yellow = deletion, red = nonsense, purple = insertion, blue = splice site, green = missense, deep red = multihit. Median variant allele fraction (VAF) per gene is shown.

B. Distribution of somatic mutations by variant allele fraction (VAF) categories

C. Germline variants in the cohort. Variant classes are color coded: red= pathogenic, blue=likely pathogenic, black=variant of unknown significance (VUS)

D. Multivariable logistic regression of factors associated with clonal hematopoiesis
Forest plot displaying odds ratios from logistic regression models evaluating the association between clonal hematopoiesis and prior radiotherapy, pathogenic/Likely pathogenic germline variants, and age at testing.

Figure 2 panel: Cardiovascular risk, clonal progression, and survival associated with clonal hematopoiesis

A. Cardiovascular Outcomes in Patients With Metastatic Prostate Cancer
Assessment of cardiovascular events including major adverse cardiovascular events (MACE) in patients with and without clonal hematopoiesis.

B. Clonal evolution over time in two patients with mCRPC demonstrating expansion of an *ATM*-mutated clone.

C. Kaplan-Meier survival analysis comparing overall survival between patients with and without CH.

Fig.1

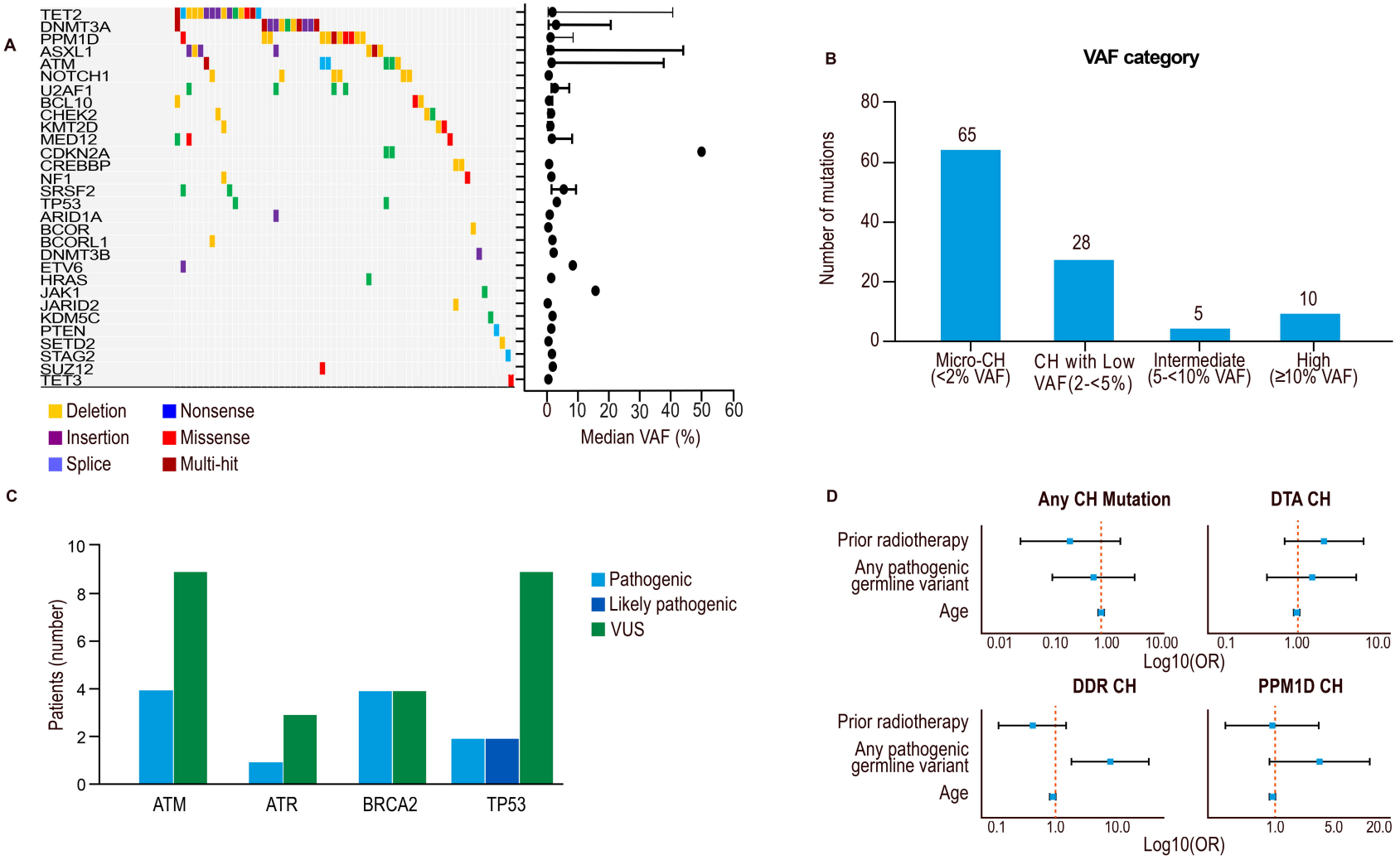
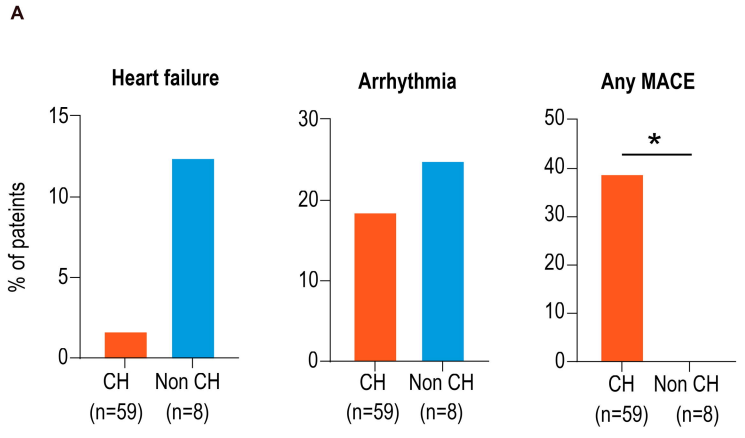
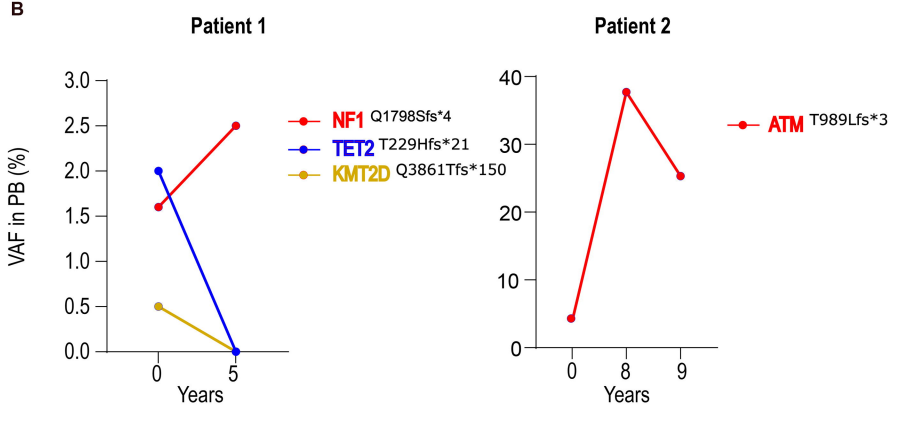
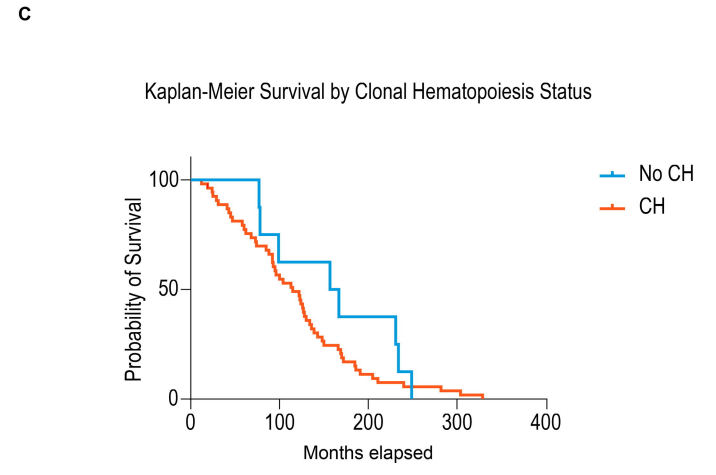

A
TET2
DNMT3A
PPM1D
ASXL1
ATM
NOTCH1
U2AF1
BCL10
CHEK2
KMT2D
MED12
CDKN2A
CREBBP
NF1
SRSF2
TP53
ARID1A
BCOR
BCORL1
DNMT3B
ETV6
HRAS
JAK1
JARID2
KDM5C
PTEN
SETD2
STAG2
SUZ12
TET3
Deletion
Insertion
Splice
Nonsense
Missense
Multi-hit
0 10 20 30 40 50 60
Median VAF (%)
B
VAF category
Number of mutations
0 20 40 60 80
65
28
5
10
Micro-CH (<2% VAF)
CH with Low VAF(2-<5%)
Intermediate (5-<10% VAF)
High (≥10% VAF)
C
Patients (number)
0 2 4 6 8 10
ATM
ATR
BRCA2
TP53
Pathogenic
Likely pathogenic
VUS
D
Any CH Mutation
DTA CH
DDR CH
PPM1D CH
Prior radiotherapy
Any pathogenic germline variant
Age
0.01 0.10 1.00 10.00
0.1 1.00 10.0
0.1 1.0 10.0
1.0 5.0 20.0
Log10(OR)

Fig.2





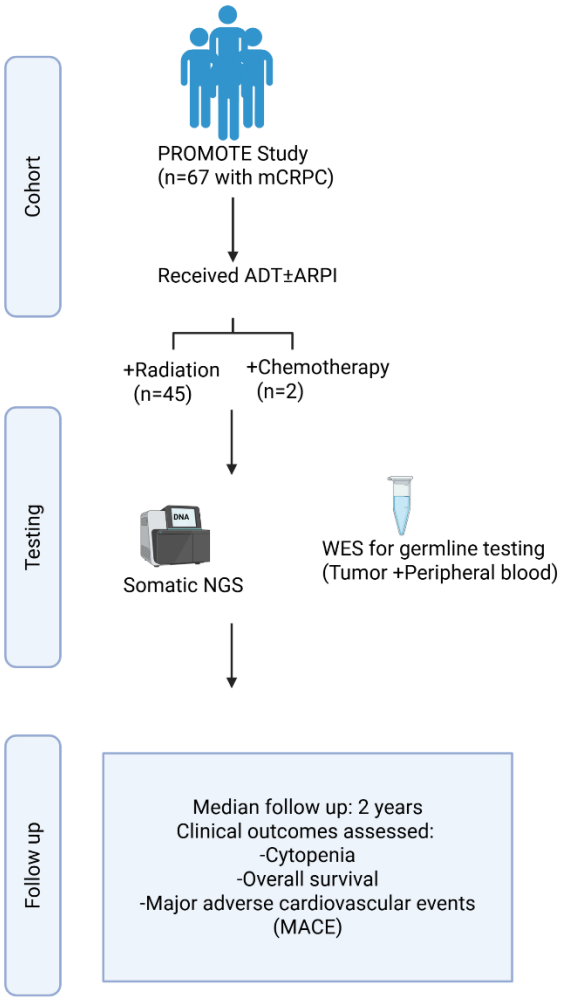


CH At risk	59	34	15	3
CH Censored	0	2	5	7
Non-CH At Risk	8	5	2	0
Non CH Censored	0	0	0	0

Inherited DNA Repair Variants are Associated with Clonal Hematopoiesis and Cardiovascular Risk in Men with Metastatic Prostate Cancer

A. Supplementary figures

Figure S1



B. Supplementary Tables

Table S1- Somatic Clonal Hematopoiesis Variants

Gene	Variant (cDNA)	Protein change	Variant Allele Frequency
ARID1A	c.2471_2472insG	S825Qfs*47	1.13%
ASXL1	c.1934dup	G646Wfs*12	1.85%
ASXL1	c.1934dup	G646Wfs*12	2.91%
ASXL1	c.2477del	G826Efs*12	3.72%
ASXL1	c.1934dup	G646Wfs*12	0.58%
ASXL1	c.2528_2529dup	T844*	0.72%
ASXL1	c.2898_2900del	G967del	44.20%
ASXL1	c.4196dup	L1399Ffs*25	0.55%
ASXL1	c.2618_2621dup	M875Sfs*6	0.80%
ASXL1	c.1934dup	G646Wfs*12	0.77%
ASXL1	c.1934dup	G646Wfs*12	23.20%
ATM	c.3285-1G>T	splice effect	1.59%
ATM	c.8494C>T	R2832C	1.72%
ATM	c.1470delT	T491Pfs*5	1.25%
ATM	c.2251-10T>G	c.2251-10T>G	37.90%
ATM	c.2965delA	T989Lfs*3	3.94%
ATM	c.8494C>T	R2832C	1.72%
ATM	c.8584+1G>T	splice effect	2.36%
BCL10	c.136del	I46Yfs*24	0.93%
BCL10	c.136del	I46Yfs*24	0.85%
BCL10	c.88G>T	E30*	1.87%
BCOR	c.1586del	N529Tfs*60	0.67%
BCORL1	c.732_736del	V245Sfs*25	1.96%
CDKN2A	c.199G>A	G67S	50.20%
CDKN2A	c.199G>A	G67S	50.20%
CHEK2	c.395del	R132Kfs*29	0.65%
CHEK2	c.591del	V198Ffs*7	1.45%
CHEK2	c.1169A>G	Y390C	1.83%
CREBBP	c.6882del	I2295Ffs*7	0.93%
CREBBP	c.4293_4297del	Y1433Gfs*2	0.90%
DNMT3A	c.1740del	W581Gfs*70	1.00%
DNMT3A	c.1474+1dup	splice effect	1.59%
DNMT3A	c.696dup	P233Afs*20	20.80%
DNMT3A	c.2711C>T	P904L	1.80%
DNMT3A	c.1240T>C	F414L	10.30%
DNMT3A	c.1238del	G413Afs*238	1.06%
DNMT3A	c.2147T>A	V716D	3.80%

DNMT3A	c.2255_2256insA	F752Lfs*5	3.13%
DNMT3A	c.1174dup	E392Gfs*3	1.77%
DNMT3A	c.1895del	K632Sfs*19	3.95%
DNMT3A	c.2054G>A	G685E	10.40%
DNMT3A	c.2043del	M682Cfs*23	0.79%
DNMT3A	c.805dup	A269Gfs*12	4.00%
DNMT3A	c.176del	P59Rfs*13	0.74%
DNMT3A	c.1903C>T	R635W	4.40%
DNMT3B	c.1329dup	V444Rfs*8	2.38%
ETV6	c.701dupC	L235Sfs*9	8.64%
HRAS	c.37G>T	G13C	1.57%
JAK1	c.1972G>A	V658I	16.00%
JARID2	c.2563del	E855Kfs*25	0.52%
KDM5C	c.3295C>A	L1099M	2.11%
KMT2D	c.11581_11582delGC	Q3861Tfs*150	1.30%
KMT2D	c.5047G>T	E1683*	1.75%
KMT2D	c.11581_11582delGC	Q3861Tfs*150	0.55%
MED12	c.6360G>C	Q2120H	8.33%
MED12	c.5815C>T	Q1939*	1.83%
MED12	c.631G>T	G211*	1.76%
NF1	c.4747G>T	E1583*	1.59%
NF1	c.5392del	Q1798Sfs*4	1.64%
NOTCH1	c.7244_7246del	P2415del	0.54%
NOTCH1	c.7244_7246del	P2415del	0.52%
NOTCH1	c.7244_7246del	P2415del	1.04%
NOTCH1	c.7244_7246del	P2415del	0.65%
NOTCH1	c.7244_7246del	P2415del	0.97%
NOTCH1	c.7244_7246del	P2415del	0.88%
PPM1D	c.1628delC	S543Lfs*4	0.84%
PPM1D	c.1601_1602delTT	F534*	1.08%
PPM1D	c.1538delT	L513*	1.32%
PPM1D	c.1636delC	L546*	2.76%
PPM1D	c.1714C>T	R572*	8.63%
PPM1D	c.1396_1399delATAC	I466Pfs*16	0.88%
PPM1D	c.1636dupC	L546Pfs*6	1.43%
PPM1D	c.1619delA	E540Gfs*7	2.14%
PPM1D	c.1717A>T	K573*	3.11%
PPM1D	c.1403C>G	S468*	1.63%
PPM1D	c.1615delG	E539Kfs*8	1.27%
PPM1D	c.1619delA	E540Gfs*7	1.30%
PTEN	c.1027-1G>T	splice effect	1.66%
SETD2	c.6358_6382del	F2120Pfs*76	0.76%

SRSF2	c.284C>A	P95H	1.67%
SRSF2	c.284C>A	P95H	9.56%
STAG2	c.1417-1G>T	splice effect	1.91%
SUZ12	c.532G>T	E178*	2.17%
TET2	c.4832delC	S1611Ffs*5	1.88%
TET2	c.5738G>A	G1913D	2.99%
TET2	c.4287dupT	G1430Wfs*48	1.83%
TET2	c.2318_2320dupGAT	S774*	2.13%
TET2	c.4633C>T	Q1545*	2.23%
TET2	c.3595-1G>T	splice effect	3.70%
TET2	c.4060dupA	R1354Kfs*47	0.59%
TET2	c.2716dupA	M906Nfs*18	0.92%
TET2	c.1965delA	K655Nfs*45	0.51%
TET2	c.4519C>T	Q1507*	2.58%
TET2	c.4990delC	Q1664Kfs*31	40.80%
TET2	c.3409+1G>A	splice effect	2.06%
TET2	c.3873G>A	W1291*	1.72%
TET2	c.1648C>T	R550*	1.76%
TET2	c.936_937delTA	T313Lfs*17	0.61%
TET2	c.5131delA	I1711Lfs*8	0.63%
TET2	c.685delA	T229Hfs*21	2.02%
TET3	c.3142G>T	E1048*	1.56%
TP53	c.818G>A	R273H	3.19%
TP53	c.646G>A	V216M	3.58%
U2AF1	c.101C>T	S34F	7.41%
U2AF1	c.470A>C	Q157P	1.57%
U2AF1	c.101C>T	S34F	2.42%
U2AF1	c.101C>T	S34F	3.05%
ZRSR2	c.399G>T	E133D	4.35%

Table S2- Pathogenic and Likely Pathogenic Germline Variants

Chromosome	Coordinates	Ref>Alt	AA change	Effect	Clinical significance	Gene
17	7578403	C>A	C165F	Non-synonymous	Likely Pathogenic	*TP53*
17	7578478	G>C	P140R	Non-synonymous	Likely Pathogenic	*TP53*
11	108143541	TC>T	.	Frameshift	Pathogenic	*ATM*
11	108143539	T>TA	.	Frameshift	Pathogenic	*ATM*
11	108218004	A>G	.	Splice site	Pathogenic	*ATM*
11	108236062	CAG>C	.	Frameshift	Pathogenic	*ATM*
13	32900280	TAA>T	.	Frameshift	Pathogenic	*BRCA2*
13	32911442	G>GA	.	Frameshift	Pathogenic	*BRCA2*
13	32914935	C>CT	.	Frameshift	Pathogenic	*BRCA2*
13	32971044	ATACT>A	.	Frameshift	Pathogenic	*BRCA2*
17	7578212	G>A	R120*	Stop gained	Pathogenic	*TP53*
17	7579393	AG>A	.	Frameshift	Pathogenic	*TP53*
3	142226847	G>A	R1589*	Stop gained	Pathogenic	*ATR*

Supplementary Figure Legend:

Figure S1: Overview of Prostate Cancer Medically Optimized Genome Enhanced Therapy (PROMOTE) Study