Replacing procarbazine with dacarbazine in escalated BEACOPP for Hodgkin lymphoma: an observational study and genomic analysis of mutational consequences for stem cells and offspring of lymphoma survivors

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Summary

Background

Procarbazine-containing chemotherapy regimens are associated with cytopenias and infertility, suggesting stem cell toxicity. When treating Hodgkin lymphoma, procarbazine in eBEACOPP (escalated dose bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone) is increasingly replaced with dacarbazine (eBEACOPDac) to reduce toxicity. The aim was to investigate the impact of this drug substitution on the mutation burden in stem cells, patient survival and toxicity.

Methods

This was a retrospective study with two co-primary objectives: to define 1) the comparative stem cell mutation burden and mutational signatures following treatment with or without procarbazine-containing chemotherapy, and 2) progression-free survival (PFS) of Hodgkin lymphoma patients treated with eBEACOPP or eBEACOPDac. Secondary objectives explored differences in specific toxicity outcomes including transfusion requirements and measures of reproductive health. We compared mutational landscapes in haematopoietic stem and progenitor cells (HSPCs) from patients treated with different Hodgkin regimens and children, sperm and bowel tissue from procarbazine-treated patients. We compared efficacy and toxicity data of a UK multicentre eBEACOPDac-treated patient cohort, with the German HD18 eBEACOPP clinical trial and UK eBEACOPP real-world datasets. Participants were adults >16 years with previously untreated Hodgkin lymphoma.

Findings

eBEACOPP-treated patients exhibit a higher burden of point mutations in HSPCs compared to eBEACOPDac and ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine)-treated patients (excess mutations: 1150 (CI_{95}=934-1366) vs 290 (CI_{95}=241-339), vs 186 (CI_{95}=116-254) respectively). Two novel mutational signatures, SBSA (SBS25-like) and SBSB were identified in HSPCs, a single neoplastic and normal colon from only procarbazine-treated patients. SBSB was also identified in germline DNA of three children conceived post-eBEACOPP and sperm of an eBEACOPP-treated male. The dacarbazine substitution did not appear to compromise efficacy. 312 eBEACOPDac patients (treated 2017-2022, 63% male, median follow-up 36.0 months (IQR=25.2-50.1)) had a 3-year PFS of 93.3% CI_{95}=90.3-96.4%, mirroring that of 1945 HD18-trial eBEACOPP patients (93.3%; CI_{95}=92.1-94.4%). eBEACOPDac-treated patients required fewer blood transfusions, demonstrated higher post-chemotherapy sperm concentrations, and experienced earlier resumption of menstrual periods.

Interpretation

Procarbazine induces a higher mutation burden and novel mutational signatures in eBEACOPP-treated patients and their germline DNA raising concerns for the genomic health of patient survivors and hereditary consequences for their offspring. However, replacing procarbazine with dacarbazine appears to mitigate gonadal and stem cell toxicity while maintaining comparable clinical efficacy.

Funding

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Introduction:

Classical Hodgkin lymphoma (cHL) is highly curable with modern therapies. Escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisolone at escalated doses; eBEACOPP) remains a gold standard first-line treatment for advanced-stage Hodgkin lymphoma conferring superior long-term progression-free survival compared with other polychemotherapy regimens(1,2). However, there are significant short- and long-term side-effects, including gonadal and stem cell toxicity. Combination chemotherapy protocols containing procarbazine have long been known to cause dose-dependent infertility(3,4), and procarbazine has been shown to have mutagenic effects in animal models(5). Replacing procarbazine with dacarbazine in COPP consolidation therapy reduced gonadal toxicity and conferred comparable long-term event-free survival in paediatric cHL(6). This successful substitution in paediatric practice encouraged adult clinicians to make the same substitution within the 7-drug eBEACOPP regimen and the resulting eBEACOPDac protocol is increasingly used in the UK and other countries despite no published data defining the efficacy or toxicity of this regimen.

While the late effects of eBEACOPP have been well documented(4,7), the mutational impact on stem cells is less well defined. Certain chemotherapy agents are known to induce specific mutational signatures in cancer cells. In the Catalogue of Somatic Mutations in Cancer (COSMIC) database(8), chemotherapy is proposed to be responsible for 7 out of 67 mutational signatures, with the aetiology of many other mutational signatures yet to be defined. Chemotherapy-induced mutagenesis has also been reported in normal somatic cells, including

colon stem cells(9) and peripheral blood cells(10). Mutational signatures caused by platinum and alkylating agents have been identified in the germline of children with paternal chemotherapy exposure prior to conception(11).

To investigate the genomic impact of cHL therapies in normal stem cells, we compared the mutational burden and signatures in the haematopoietic stem and progenitor cells (HSPCs) of long-term remission patients previously treated with doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD), eBEACOPP or escalated BEACOPDac (eBEACOPDac) where procarbazine was replaced by dacarbazine. We then defined the extent of chemotherapy-induced mutagenesis in other stem cell compartments and in tumour and normal tissues from one patient. To study the impact of eBEACOPP on both maternal and paternal gonadal stem cells, we mapped mutational signatures and burdens in 5 children of a female patient (two conceived pre- and three post-eBEACOPP) as well as in sperm from a male donor treated with eBEACOPP.

To validate the clinical efficacy of the eBEACOPDac regimen we collected multicentre data on eBEACOPDac-treated patients and compared clinical outcomes with two independent eBEACOPP datasets: the German HD18 trial to compare treatment efficacy and maximise statistical power, and a real-world UK eBEACOPP dataset to compare specific toxicity outcomes.

Methods:

Study design and datasets

This was a retrospective study comparing genomic and clinical consequences of treating Hodgkin lymphoma patients with or without procarbazine-containing chemotherapy.

For the stem cell mutational studies, patients donated blood for research to the Cambridge Blood and Stem Cell Biobank (CBSB), which has Health Research Authority and NHS Research Ethics Committee approval (REC reference 18/EE/0199, IRAS 149581). Peripheral blood mononuclear cells (PBMC) were isolated from 12 advanced-stage Hodgkin lymphoma patients who had been in remission for ≥6 months (appendix p12). The patients had been previously treated with either eBEACOPDac (n=4), eBEACOPP (n=5) or ABVD (n=3).

The clinical dataset was a retrospective study of 312 adults aged >16 years with advanced-stage cHL, treated with first-line escalated BEACOPDac at 25 centres in the UK, Ireland and France. The study was conducted with Health Research Authority (REC reference 20/HRA/3762, IRAS 278806) and Public Benefit and Privacy Panel approval.

We compared survival outcomes to 1945 adults (aged 18-60 years) treated with first-line eBEACOPP in the randomised HD18 trial. Toxicity outcomes were compared with a retrospective UK dataset of 73 adults aged >16 years treated with first-line eBEACOPP.

Procedures

PBMCs were cultured for 14 days and single-cell derived HSPC colonies were harvested. DNA was extracted from each colony and 6-8 single-cell derived HSPC colonies were whole-genome sequenced from each of the 12 patients (n=91; mean sequencing depth 26X).

CaVEMan (Wellcome Sanger Institute, Cambridge, UK) (12), used for calling single nucleotide variants (SNVs), was run against an unmatched synthetic normal genome. Insertions and deletions (indels) were identified using the Pindel algorithm applied to a matched normal sample.

The linear regression of age and SNV or indel mutation burden(13) from a control cohort (n=110; mean sequencing depth 24X) was used as a baseline against which to compare the mutation burden of the chemotherapy-exposed individuals. The mutation burden confidence intervals were calculated using the output from the linear mixed models performed in R 4.2.2.

Whole-genome sequencing (WGS) was performed on a caecal adenocarcinoma from a Hodgkin lymphoma survivor treated with Chlorambucil, Vinblastine, Procarbazine, Prednisolone (ChlVPP) nine years before sampling(14). Previously published(14) normal colorectal epithelium from the same individual was also interrogated.

Buccal DNA was obtained from five children and the spouse of a cHL female patient (who was treated with eBEACOPP prior to conceiving her 3rd child) and subjected to WGS (appendix p2-3). Mutational burden and signatures were compared between pre- (n=2) and post-chemotherapy children (n=3).

The sperm DNA of a patient with mild oligospermia (13M/ml) 3.5 years post 4 cycles of eBEACOPP treatment was sequenced using Nanoseq WGS (Wellcome Sanger Institute, Cambridge, UK) (15) (appendix p3-4). To establish a control cohort for comparison, the parental germline de novo point mutation burden (DNM) was predicted based on pedigree studies from parents and offspring trio WGS(16).

Treatment with eBEACOPP has been previously described(17). eBEACOPDac is a modified version of the eBEACOPP protocol, in which oral procarbazine is removed and replaced with intravenous dacarbazine (250mg/m^2 D2-3) (appendix p13).

Progression-free survival (PFS) of the 312 eBEACOPDac-treated patients was compared with 1945 eBEACOPP-treated HD18 trial patients(17), through collaboration with the German Hodgkin Study Group (GHSG). 265 (85%) of 312 eBEACOPDac patients were treated using

an ‘HD18-like’ approach(17), namely those with a metabolic remission on interim PET-CT after 2 cycles (iPET2) received 2 additional cycles of eBEACOPDac, and those with positive iPET2 received 4 additional cycles of eBEACOPDac. iPET2 Deauville score of 3 or less was considered a complete metabolic remission. 47 (15%) of 312 patients followed the AHL2011 protocol (18) with 2 cycles of eBEACPODac given upfront and if iPET2 negative were deescalated to 4 cycles of ABVD.

To refine our comparative analysis, we used propensity score matching (PSM) based on age, sex, stage, and international prognostic score (IPS, potential range: 0-7) to match HD18-like patients with a subgroup of HD18 trial patients. Sex (male/female) was defined as per the medical records. PFS was calculated from the date of diagnosis to the date of relapse, death, or last follow-up (see appendix p18 for reasons for censoring). Survival analyses were performed using the Kaplan-Meier method. Pre-PSM hazard ratios (HR) were obtained from Cox regression adjusted for IPS, age and sex, while post-PSM HRs were obtained from Cox regression analysis adjusted for the propensity score.

Toxicity outcomes of the eBEACOPDac cohort were compared with a real-world eBEACOPP cohort of 73 patients treated at 7 UK centres. Toxicity data were only collected from patients who had received ≥4 cycles of chemotherapy (261 eBEACOPDac patients and 72 eBEACOPP patients), in order to match known confounding variables and limit bias.

Outcomes

This was a retrospective study with two co-primary objectives to define: 1) the comparative stem cell point mutation and indel burden as well as mutational signatures following treatment with or without procarbazine-containing chemotherapy and 2) PFS of patients treated with eBEACOPP or eBEACOPDac. Secondary objectives were to define differences

in specific toxicity outcomes: day 8 (D8) neutrophil count, D8 alanine transaminase (ALT), units of red cells transfused, days of non-elective hospital admission, time to return of menstrual periods in women <35 years, spermiogram data pre-and >2 years post-chemotherapy.

While the duration of follow-up has allowed comparison of survival outcomes, and the above toxicities, eBEACOPDac has not yet been in use long enough for data collection on longer term toxicities such as the incidence of second cancers and birth rate after chemotherapy.

Statistical analysis

The Hierarchical Dirichlet process (HDP) was employed to extract mutational signatures from SBS/Indels derived from HSPCs, germline DNMs from the offspring of an eBEACOPP-treated female, and mutations from the sperm of an eBEACOPP-treated male (appendix p4-5).

Clinical statistical analyses were performed using R 4.2.2 software and SPSS V.28. No power calculation was performed for this retrospective study. When comparing toxicity data, the two-sided Mann-Whitney U test and t-test were used for continuous variables and Fisher's exact test for categorical variables. The results are presented in Table S4 (appendix p15). A p-value of <0.05 was considered statistically significant. Patients were excluded if they had missing data points for the variable under analysis.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results:

We found a linear correlation between both the number of SNV and indel burdens with age across all cohorts (Figure 1A and appendix p14, Pearson correlation coefficient for normal population 0.985 for SNV, 0.908 for indels). In line with previous studies, normal adult HSPCs accumulated ~18 SNVs per year post birth(13). Consequently, the mutation burden increases from 400 SNVs in 20-year-old individuals to nearly 1500 SNVs at the age of 80 years. Additionally, HSPCs from all chemotherapy-exposed individuals demonstrated higher than expected mutation loads for their respective ages. ABVD-treated patients and eBEACOPDac-treated patients had a minor excess somatic mutation burden of 186 (CI_{95}=116-254) and 290 (CI_{95}=241-339), respectively, compared to age-matched normal HSPCs. However, we observed significant elevations in SNV burdens in patients receiving eBEACOPP treatment, with 1150 (CI_{95}=934-1366) excess mutations. A similar pattern was also observed while analysing small indels in HSPCs. There was a comparative indel mutation burden of -0.4 (CI_{95}=-2.9-2.1) in ABVD-treated patients, 11.2 (CI_{95}=6.9-15.6) in eBEACOPDac-treated patients and 64.8 (CI_{95}=53.1-76.5) in eBEACOPP-treated patients (appendix p6).

The post-chemotherapy children had a modest but significant number of de novo germline point mutations (DNMs). After correcting for the paternal component of DNMs, we observed a significant increase (~2.3 times; appendix p11) in the maternally inherited DNMs among the post-chemotherapy children (p-value 0.025; t-test; Figure 2C). Moreover, analysis of sperm DNA of a patient post-eBEACOPP revealed a 3-times higher mutation burden, after correcting for his age, compared to the control cohort(16) (Figure 2D).

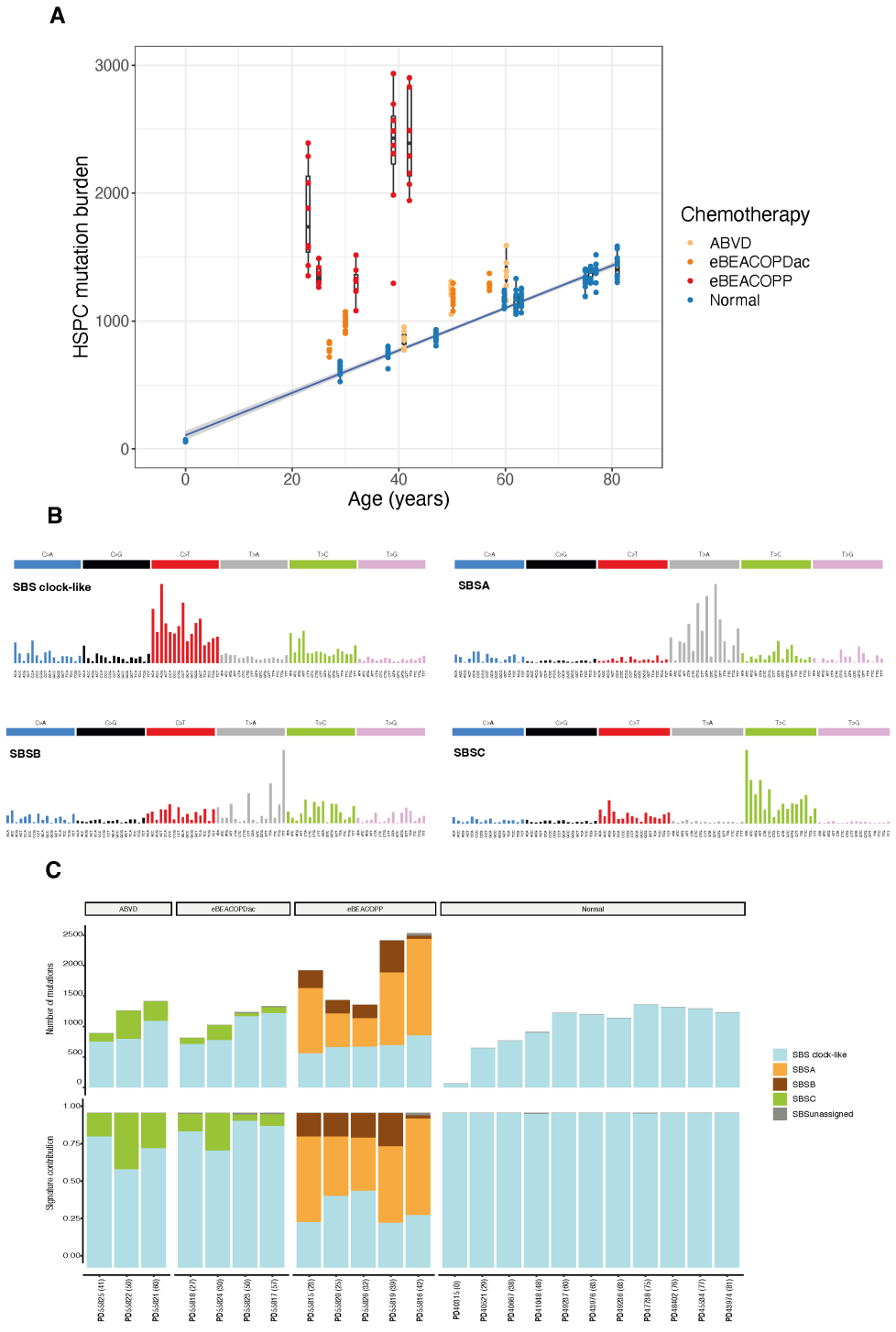


Figure 1: **(A)** Burden of HSPC single nucleotide variants across the chemotherapy donor and a comparable normal donor cohort. The boxplots represent data from individual HSPC colonies (n = 201; 6-10 colonies per individual) and are coloured by chemotherapy exposure. The boxes indicate the median and interquartile range and the whiskers denote the range. The blue line represents a regression of age on mutation burden for the normal donors, with 95% CI shaded in grey. **(B)** Mutational signatures extracted using HDP. **(C)** Proportion of extracted signatures active in eBEACOPP, cBEACOPDac, ABVD and normal individuals.

HSPC = Haematopoietic stem and progenitor cells, HDP= Hierarchical Dirichlet process

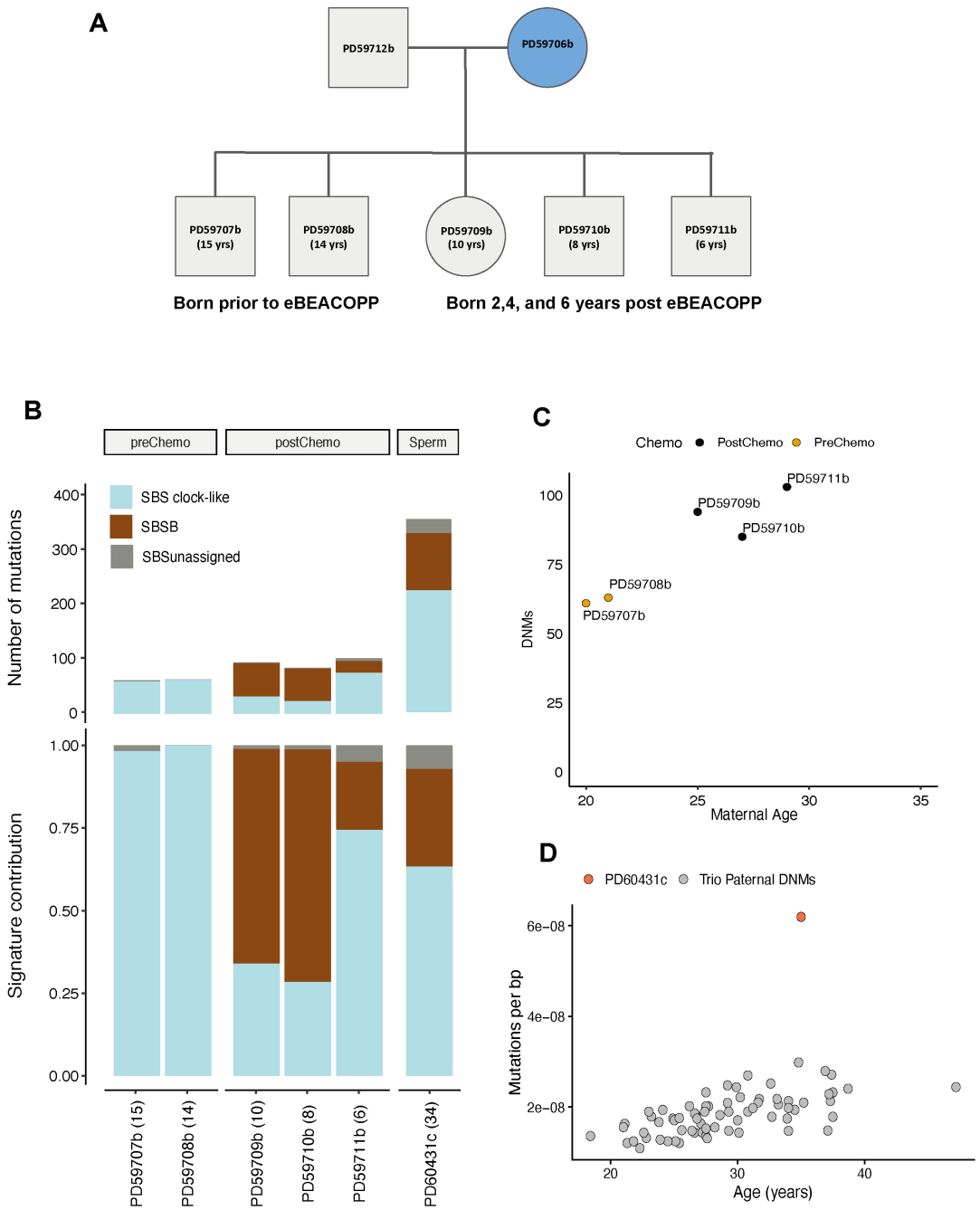


Figure 2: **(A)** Pedigree of female Hodgkin lymphoma patient who received 6 cycles eBEACOPP. 2 children were born pre-chemotherapy, and 3 children were conceived post-chemotherapy. **(B)** SBS mutational signature contribution in pre- and post-chemotherapy children and sperm sample from the patient treated with 4 cycles eBEACOPP. **(C)** Number of *de novo* mutations in pre- and post-chemotherapy children plotted against maternal age (p-value 0.011). P-value was generated using t-test to check significance of the pre-chemo probands burden to the post-chemo probands. **(D)** Mutation burden in sperm of oligospermic patient treated with 4 cycles eBEACOPP compared with paternal mutation burden in trio studies[10].

DNMs = De novo mutations. SBS = single base substitution. Bp = base pair

Four SNV mutational signatures were extracted from HSPCs, namely: SBS clock-like, SBSA, SBSB, and SBSC (Figure 1C). Other than SBS clock-like signature, the remaining three signatures are novel and have not been reported in the COSMIC database. SBS clock-like is a combination of SBS1, associated with the deamination of 5-methylcytosine, SBS5 of unknown aetiology, and 'SBSBlood', the predominant 'clock-like' signature present in HSPCs. SBSA is only observed in eBEACOPP treated patients and shares 0.84 cosine similarity to COSMIC v3.4 signature SBS25. Similarly, SBSB, also with predominant T>A substitutions, is found in all patients exposed to eBEACOPP, but with smaller contributions. These two mutational signatures have been recently ascribed to procarbazine treatment(19,20). SBSC was detected in patients treated with either ABVD or eBEACOPDac (Figure 1C). The sole shared chemotherapy agent in these protocols is Dacarbazine, implying an association between this drug and SBSC. Specific SBS mutational signatures were not obviously associated with topoisomerase inhibitors, vinca-alkaloids or bleomycin.

Two indel signatures were extracted, of which one signature was a combination of the COSMIC v3.4 indel clock-like signatures. The second signature was a novel signature that was observed only in eBEACOPP-treated patients and recently attributed to procarbazine(19) (appendix p6).

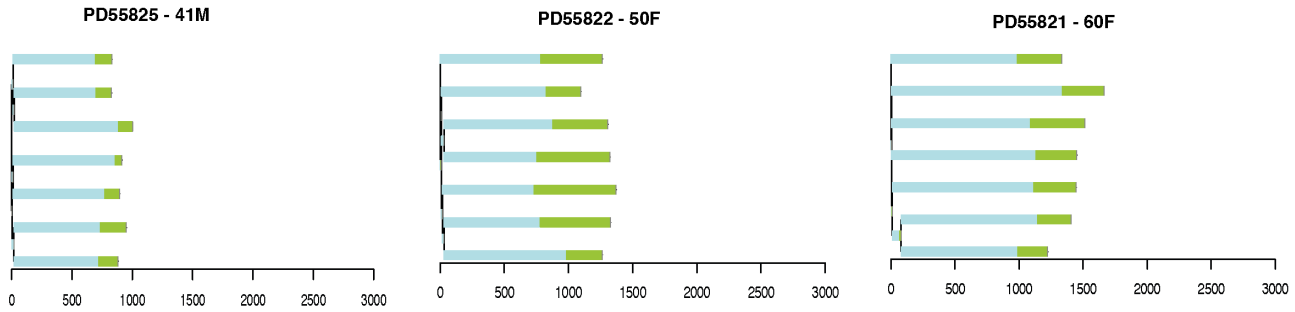
Mutational signatures were extracted from the five children of the eBEACOPP-treated female. SBS clock-like signature was extracted from all the children, consistent with the previously reported mutational signatures in germline DNMs(21). Additionally, the procarbazine-associated signature SBSB, was identified in all three children conceived post-chemotherapy and was absent in the two children born prior to chemotherapy.

Intriguingly, mutational signature analysis of sperm from the eBEACOPP-treated male revealed that approximately one third of mutations were attributed to SBSB (Figure 2B). The remaining mutations were mapped to SBS clock-like signatures as previously reported(15,22).

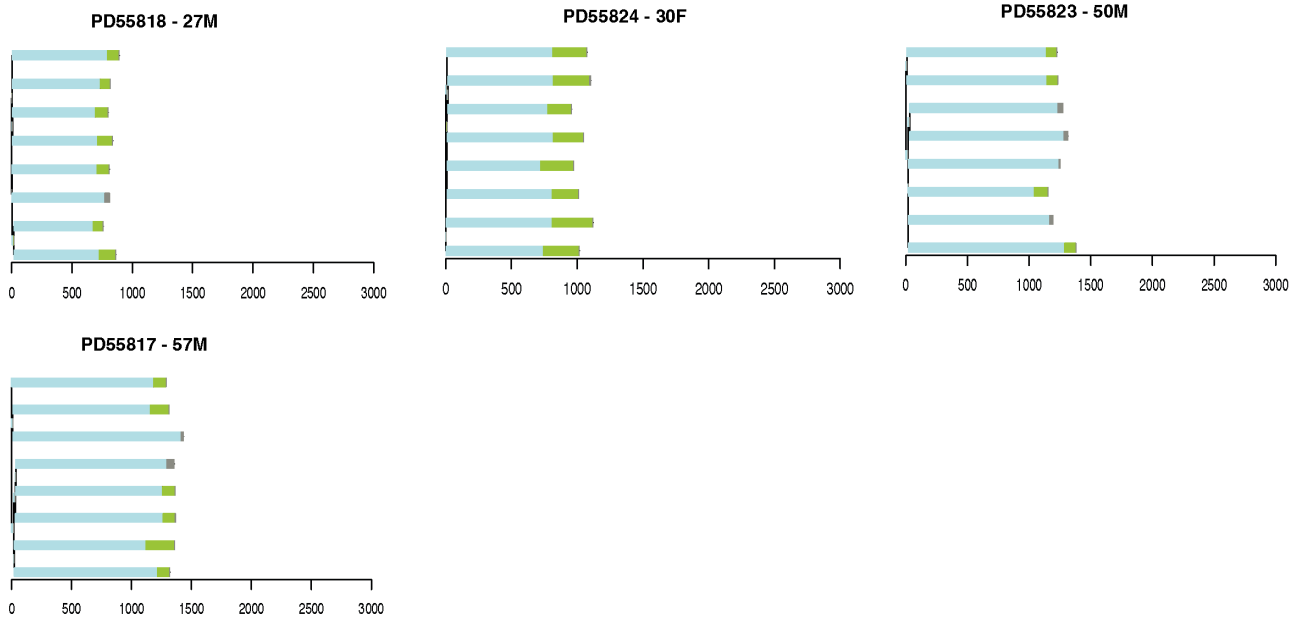
To investigate whether ABVD, eBEACOPDac, or eBEACOPP exert consistent effects across multiple HSPC colonies, we examined the HSPC phylogenetic trees from all donors (Figure 3). The dacarbazine-associated signature, SBSC, was identified in all HSPC colonies of three ABVD-treated individuals and in 50-100% of colonies of four eBEACOPDac-treated individuals. In eBEACOPP-treated patients, the procarbazine-associated signatures, SBSA and SBSB, were jointly detected in all colonies, except in patient PD55816, where the signatures co-occurred on only two branches. HSPC colonies from normal donors only exhibit SBS clock-like signature (appendix p7).

WGS of colon from a procarbazine-exposed patient revealed a significant burden of the procarbazine-associated signature, SBSA, in both normal colonic crypts and cancer micro-biopsies (appendix p8). There is no increased frequency of SBSA in subclonal crypts relative to adjacent normal crypts, suggesting that mutations bearing this signature likely originated from a colorectal stem cell or occurred during early stages of tumorigenesis. These findings imply a long-term impact of procarbazine on normal solid tissues, beyond haematological effects.

ABVD



eBEACOPDac



eBEACOPP

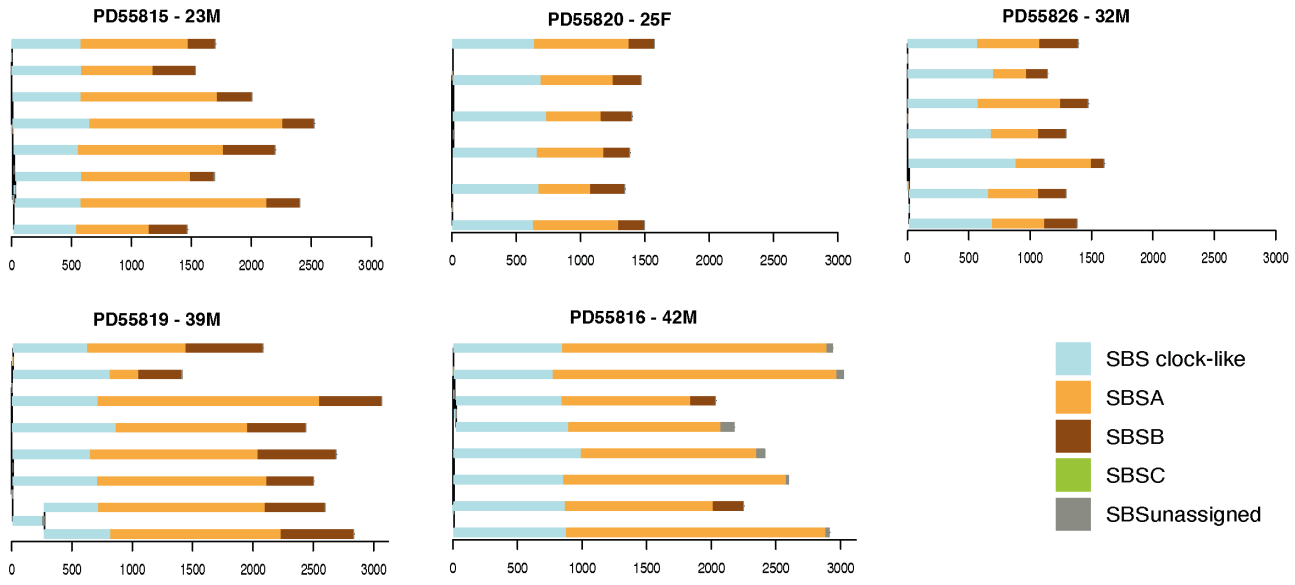


Figure 3: Phylogenetic trees with assigned mutational signatures from individuals treated with ABVD, eBEACOPDac or eBEACOPP. Individuals are ordered by age. Branch lengths are proportional to the mutation count. The mutational signatures contributing to each branch are colour coded as indicated at the bottom right. “SBSunassigned " indicates mutations that are not confidently attributed to any signature.

The dacarbazine-associated signature, SBSC, was identified in all HSPC colonies of three ABVD-treated individuals. In BEACOPDac-treated patients, the presence of SBSC was observed in all colonies from patient PD55824, in 7/8 colonies from PD55818, in 6/8 colonies from PD55817, and in 4/8 colonies from PD55823. In eBEACOPP-treated patients, the procarbazine-associated signatures, SBSA and SBSB, were jointly detected in all colonies, except in patient PD55816, where the signatures co-occurred on only two branches.

Between 1st January 2017 and 31st December 2022, 312 patients with advanced-stage cHL were treated with first-line eBEACOPDac at 25 centres in the UK, Ireland and France (appendix p15). Their survival outcomes were compared with 1945 patients treated with eBEACOPP in the HD18 trial(17) between 14th May 2008 and 18th July 2014. Using propensity score matching (PSM), 248 eBEACOPDac-treated HD18-like patients were matched with 248 patients treated with eBEACOPP in the HD18 trial. Patients were well-matched for age, sex, stage and IPS (Table 1). No data on race/ethnicity were collected. eBEACOPDac-treated patients received fewer cycles of chemotherapy than HD18 patients (median: 4 vs 6). 14 (5.7%) of 246 of the eBEACOPDac cohort received radiotherapy compared to 71 (28.6%) of 248 of the HD18 cohort. Median follow-up was 36 months (IQR 25.2-50.1; eBEACOPDac) and 57 months (IQR 35.4-64.7; HD18).

The 3-year PFS of the eBEACOPDac cohort mirrors the HD18 3-year PFS both before PSM (93.3% (CI_{95}=90.3-96.4%) vs 93.3% (CI_{95}=92.1-94.4%)) and after (92.1% (CI_{95}=88.5-95.8%) vs 91.7% (CI_{95}=88.1-95.3%)) (Figure 4). 3-year overall survival of the eBEACOPDac cohort is 99.3% (CI_{95}=98.4-100%) (appendix p9).

Of 312 patients who started eBEACOPDac, 3 patients had primary refractory disease, and 14 have relapsed at 6 to 60 months. 13 of 17 patients with relapse/refractory disease are currently in remission following subsequent treatment including haematopoietic stem cell transplantation (appendix p10). One 56-year-old patient died with bowel perforation during cycle 1 eBEACOPDac, one 30-year-old died during allogeneic stem cell transplantation for relapsed disease, one 34-year-old with alcoholic liver disease died in remission 8 months after eBEACOPDac, and one 44-year old died from suicide while in remission 3 years post-treatment for relapsed cHL.

From 1st January 2009 to 31st December 2022, 73 patients with advanced-stage cHL were treated with first-line eBEACOPP at 7 UK centres. Toxicity was compared between the eBEACOPDac cohort (n=312) and the real-world UK eBEACOPP cohort (n=73) over the first 4 cycles (appendix p16). eBEACOPP and eBEACOPDac patients were well-matched with no significant differences in age, sex, or stage (stage 3-4: 58 (79%) of 73 vs 260 (83%) of 312), although the eBEACOPP patients had higher risk disease (IPS≥3 in 56 (77%) of 73 vs 194 (62%) of 311; p=0.021). 178 (57%) of 312 eBEACOPDac patients received only 4 cycles (vs 12 (16%) of 73 eBEACOPP patients; p<0.0001), as the eBEACOPDac cohort was largely treated after publication of HD18 trial data(17). Most real-world eBEACOPP patients were treated with an HD15 approach(23) (6 cycles of escalated BEACOPP followed by PET-guided radiotherapy).

When comparing patients who had received ≥4 cycles in the eBEACOPDac (n=261) versus the eBEACOPP cohort (n=72), there was no significant difference in mean D8 ALT (46.0 (SD 29.2) vs 38.8 (SD 29.5); p=0.081); or in mean D8 neutrophil count (2.55 (SD 2.18) vs 3.00 (SD 1.95) ; p=0.13) in patients given granulocyte-colony stimulating factor from D9.

There were fewer non-elective days of inpatient care for eBEACOPDac patients compared to eBEACOPP patients (mean 3.23 (SD 5.96) vs 5.23 (SD 7.23); p=0.031). However, as eBEACOPDac patients were treated more recently there may be some era effect. Importantly, eBEACOPDac patients received fewer red cell transfusions compared to eBEACOPP patients (mean 1.70 (SD 2.77) vs 3.69 (SD 3.89); p<0.0001). Of the women aged <35 years who completed ≥4 cycles of chemotherapy, 65 (100%) of 65 had a return of menstrual periods after eBEACOPDac compared to 25 (89%) of 28 after eBEACOPP. eBEACOPDac patients appeared to restart menstruation earlier post-chemotherapy (mean 5.04 (SD 3.07) vs 8.77 (SD 5.57) months; p=0.0036). However, eBEACOPP patients received more cycles of chemotherapy. The use of Goserelin to suppress ovulation varied between centres.

eBEACOPDac and eBEACOPP-treated patients had similar sperm concentrations pre-treatment (median 22.5 (IQR 0.2-75.7) vs 27.5 (14.9-46.0) Million/ml, p=0.68; Figure 4C). However, >2 years post-chemotherapy, there was a striking difference between the two cohorts and 6 (85%) of 7 eBEACOPDac-treated males (median age 21 years, IQR 20-25) had a normal sperm concentration, while 6 (85%) of 7 eBEACOPP-treated patients (median age 32 years, IQR 26-43) were azoospermic (median 23.4 (IQR 11.0-32.3) vs 0.0 (0.0-0.001) Million/ml; p=0.0040; Figure 4D). Sperm motility was also normal in the 7 eBEACOPDac-treated males, but the proportion of sperm with normal morphology was low (median=2.5% (IQR 2.0-3.4); appendix p17).

<u>Table 1:</u> Baseline characteristics (after propensity score matching) of escalated BEACOPDac patients (n=248) versus HD18 trial escalated BEACOPP patients (n=248)

Patient Characteristics	Escalated BEACOPDac (after PSM) N=248		HD18 Escalated BEACOPP (after PSM) N=248	
Median Age, years (IQR)	25.5 (21-32.5)		25 (22-33)	
Age ≤ 45 years Age >45 years	229 (92.3%) 19 (7.7%)		225 (90.7%) 23 (9.3%)	
Male sex (%)	157 (63.3%)		157 (63.3%)	
Stage II III IV	41 (16.5%) 44 (17.7%) 163 (65.7%)		26 (10.5%) 65 (26.2%) 157 (63.3%)	
IPS 0-2 IPS >2	93 (37.5%) 155 (62.5%)		91 (36.7%) 157 (63.3%)	
Median number of cycles, (range)	4 (1-6)		6 (1-8)	
Number of cycles*	iPET2 pos	iPET2 neg	iPET2 pos	iPET2 neg
1-3 4 5 6 7-8	2 (3.3%) 0 (0.0%) 3 (5.0%) 55 (91.7%) 0 (0.0%)	2 (1.0%) 167 (90.3%) 2 (1.0%) 14 (7.6%) 0 (0.0%)	3 (2.1%) 0 (0.0%) 1 (0.7%) 133 (93.7%) 5 (3.5%)	1 (1.0%) 50 (50.5%) 1 (1.0%) 47 (47.5%) 0 (0.0%)
N	60	185	142	99
Radiotherapy	14/246 (5.7%)		71 (28.6%)	
Progressive disease/relapse	16 (5.8%)		21 (8.5%)	
Death	4 (1.6%)		3 (1.2%)	
Median follow-up in months (IQR)	36.0 (25.2-50.1)		57.3 (35.4-64.7)	

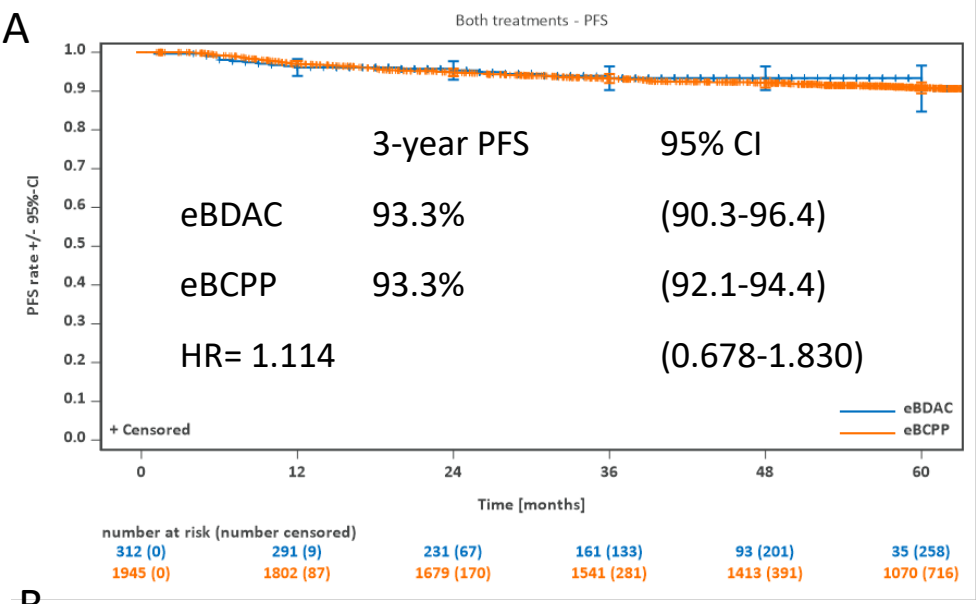
PSM = Propensity score matching, IPS = International Prognostic Score.

*eBEACOPDac patients: iPET2 negative = DS 1-3.

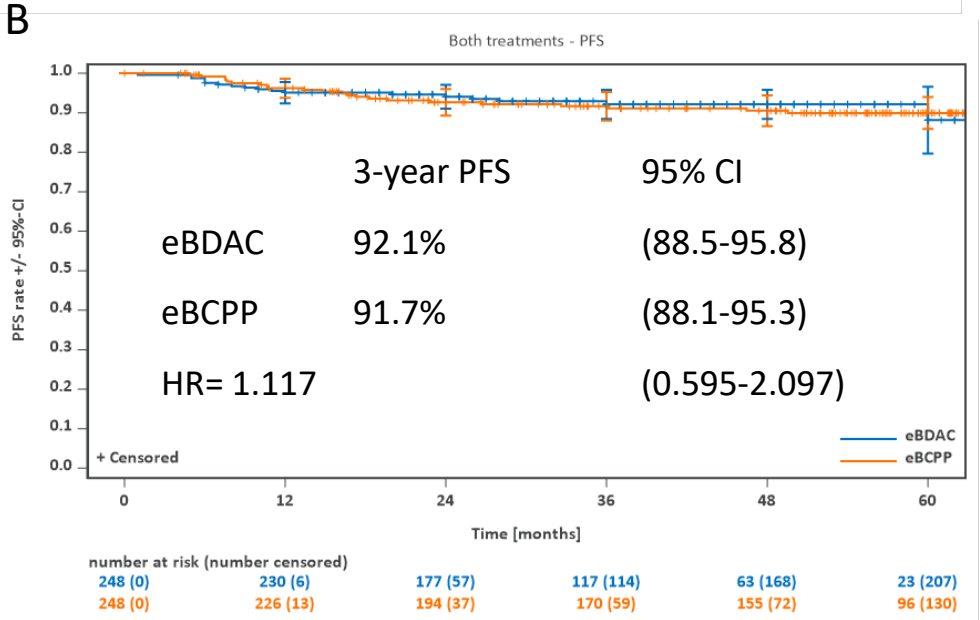
eBEACOPP HD18 patients: iPET negative = DS 1-2. Note HD18 iPET2 negative patients were randomised to complete 4 or 6 cycles of eBEACOPP in total.

IQR=interquartile range

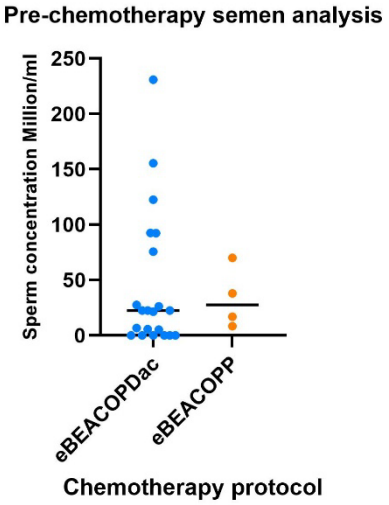
A


Both treatments - PFS
PFS rate +/- 95%-CI
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
3-year PFS
95% CI
eBDAC
93.3%
(90.3-96.4)
eBCPP
93.3%
(92.1-94.4)
HR= 1.114
(0.678-1.830)
+ Censored
eBDAC
eBCPP
0
12
24
36
48
60
Time [months]
number at risk (number censored)
312 (0)
291 (9)
231 (67)
161 (133)
93 (201)
35 (258)
1945 (0)
1802 (87)
1679 (170)
1541 (281)
1413 (391)
1070 (716)

B


Both treatments - PFS
PFS rate +/- 95%-CI
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
3-year PFS
95% CI
eBDAC
92.1%
(88.5-95.8)
eBCPP
91.7%
(88.1-95.3)
HR= 1.117
(0.595-2.097)
+ Censored
eBDAC
eBCPP
0
12
24
36
48
60
Time [months]
number at risk (number censored)
248 (0)
230 (6)
177 (57)
117 (114)
63 (168)
23 (207)
248 (0)
226 (13)
194 (37)
170 (59)
155 (72)
96 (130)

C


Pre-chemotherapy semen analysis
Sperm concentration Million/ml
250
200
150
100
50
0
eBEACOPDac
eBEACOPP
Chemotherapy protocol

D

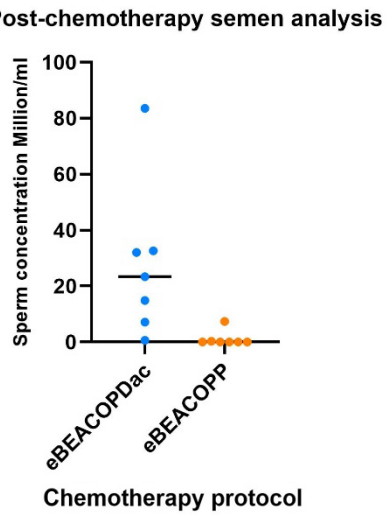

Post-chemotherapy semen analysis
Sperm concentration Million/ml
100
80
60
40
20
0
eBEACOPDac
eBEACOPP
Chemotherapy protocol

Figure 4: **(A)** Kaplan-Meier estimates of progression-free survival of the escalated BEACOPDac-treated patients (n=312) compared to the HD18 trial escalated BEACOPP-treated patients (n=1945) before propensity score matching. **(B)** Kaplan-Meier estimates of progression-free survival of the escalated BEACOPDac-treated patients (n=248) compared to the HD18 trial escalated BEACOPP-treated patients (n=248) after propensity score matching. The hazard ratio was obtained from Cox regression adjusted for propensity score. The proportional hazards assumption was tested using the Schoenfeld residuals test and confirming by visual inspection. For the Cox regression comparing the 1945 HD18 vs 312 eBEACOPDac patients (predictors: trial, IPS, age, sex) the proportional hazards assumption was not met. For the Cox regression of the propensity score matched comparison (248 vs 248 patients; predictors; trial, PS) on the other hand, the proportional hazards assumption could be verified. **(C)** Sperm concentration of Hodgkin lymphoma patients prior to treatment with escalated BEACOPDac (n=21) or eBEACOPP (n=4) chemotherapy. **(D)** Sperm concentration >2 years following completion of eBEACOPDac (n=7) or eBEACOPP (n=7) chemotherapy.

eBPDac = escalated BEACOPDac, eBCPP = escalated BEACOPP, HR = Hazard ratio, PFS = progression-free survival, 95% CI = 95% Confidence interval

Panel: **Research in context**

Evidence before this study:

The multinational randomised EuroNet-PHL-C1 trial showed that replacing procarbazine with dacarbazine in COPP consolidation therapy reduced gonadal toxicity and conferred comparable long-term event-free survival in paediatric classical Hodgkin lymphoma. This successful substitution in paediatric practice encouraged adult clinicians to make the same substitution within the 7-drug eBEACOPP regimen. The resulting eBEACOPDac protocol is increasingly used in the UK and other countries despite no published data defining the efficacy or toxicity of this regimen. We searched PubMed between 1st January 1998 and 31st August 2024 using the terms "BEACOPP" and "dacarbazine" however there are no publications on this topic.

Added value of the study:

Our study shows that replacing procarbazine with dacarbazine in eBEACOPP is unlikely to compromise the efficacy of treatment. 3-year progression-free survival of 312 eBEACOPDac patients (93.3%; CI_{95}=90.3-96.4%) mirrored that of 1945 HD18-trial eBEACOPP patients (93.3%; CI_{95}=92.1-94.4%). eBEACOPDac-treated patients required fewer blood transfusions, demonstrated higher post-chemotherapy sperm concentrations, and experienced earlier resumption of menstrual periods.

Mutational analysis of HSPCs revealed a grossly elevated mutation burden in eBEACOPP-treated patients as well as two novel mutational signatures (SBSA (SBS25-like), and SBSB). Analysis of sperm and children of procarbazine-treated patients demonstrates that SBSB, but not SBSA, is inherited in the germline.

Implications of all the available evidence:

There is strong supportive evidence that eBEACOPDac is highly efficacious Hodkgkin lymphoma therapy. The clear benefits in terms of stem cell genomic health and fertility provide even more reason for clinicians offering frontline eBEACOPP to consider replacing procarbazine with dacarbazine. This drug substitution is easily accomplished in routine clinical practice, can be implemented rapidly with minimal resource implications, and constitutes and immediate means to reduce treatment toxicity and improve genomic and reproductive health in young Hodgkin lymphoma patients across the world.

Discussion:

To our knowledge, this is the first study demonstrating the impact of lymphoma polychemotherapy on genomic health of normal somatic tissues, tumour and the germline.

The SBS25 mutational signature has previously been described in two Hodgkin lymphoma cell lines from patients exposed to chemotherapy(24), and has also been identified in cell-free DNA of treated cHL patients(25). A recent study demonstrating SBS25 in relapsed cHL suggested a link with procarbazine/dacarbazine(26). Our study has demonstrated that SBSA (SBS25-like), SBSB, and a novel indel signature are caused by procarbazine and contribute to the large excess mutation burden in the HSPCs of eBEACOPP-treated patients.

We have observed an increased number of de novo mutations and the procarbazine-associated signature, SBSB, in the sperm of an eBEACOPP-treated male, and in the germline DNA of children conceived after maternal procarbazine exposure. Although the patient numbers are small for generalisation, this observation marks the first direct detection of a chemotherapy signature in sperm, underscoring the potential long-term impact of treatment on germline DNA integrity. However, currently the duration of these effects in sperm and their potential implications for fertility are unknown. Our findings align with previous reports of chemotherapy-induced signatures in offspring(11) and highlight the need for continued research into the hereditary implications of chemotherapy treatment.

cHL survivors are known to be at risk of treatment-related second cancers. A large Dutch registry study has shown that procarbazine-containing chemotherapy increases the risk of gastrointestinal cancers, non-Hodgkin lymphoma and leukaemia/MDS (HR 2.7, 3.9, 1.2, respectively) compared with no chemotherapy(7). The consequences of procarbazine-induced mutational burden and signatures in normal tissues remain unknown, however in some contexts procarbazine can cause the acquisition of driver mutations. Recent data from paediatric cHL

survivors suggest that SBS25 is the likely cause of the *STAT3 Y640F* mutation, which has previously been identified as a gain-of-function driver mutation in T-cell large granular lymphocytic leukaemia(27). Our data indicate that SBS25 is not confined to HSPCs in procarbazine-exposed patients but is also induced in other stem cell compartments, including colonic mucosa(14). However, as the driver mutations in this cHL survivor's caecal adenocarcinoma were in the C>T context (in *KRAS* and *APC*), and not in the T>A context, the role of SBS25 in this malignancy remains unclear.

Although not a prospective non-inferiority study, our data strongly suggest that replacing procarbazine with dacarbazine is unlikely to compromise the efficacy of eBEACOPP. There is no discernible difference in the 3-year PFS of the eBEACOPDac cohort and the HD18 trial cohort when comparing whole cohorts or in the sensitivity analysis, which matched 248 UK patients with 248 HD18 trial patients following PSM. The data also indicate that replacing procarbazine with dacarbazine has toxicity benefits. Although the data are retrospective, and do not detail CTCAE grading, they reveal a marked reduction in blood transfusion requirement in patients receiving eBEACOPDac compared to eBEACOPP, a finding that is mirrored closely in the prospective randomised HD21 trial, where patients treated with the procarbazine-free arm, BrECADD (brentuximab vedotin, etoposide, cyclophosphamide, doxorubicin, dacarbazine, dexamethasone), had a reduced blood transfusion requirement compared with eBEACOPP-treated patients (28).

Although only a minority of cases were studied for male gonadal toxicity, there appears to be a clear reduction in gonadal toxicity in patients treated with eBEACOPDac compared to those receiving eBEACOPP. Sperm concentration was normal >2 years post-chemotherapy in most eBEACOPDac-treated males, whereas there was a high incidence of azoospermia and oligospermia after eBEACOPP. These findings are consistent with the previously published comparative study of mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) vs

ABVD(29), GHSG data(30) and the EuroNet PHL C1 trial where the same drug substitution was made(6). A return of menstrual periods was observed in all eBEACOPDac-treated females <35y and this occurred significantly earlier than in the real-world eBEACOPP cohort. Previous studies of reproductive patterns among Scandinavian cHL survivors have shown that males and females who receive ABVD have higher fertility rates than matched controls(31), and 6-8 cycles eBEACOPP is associated with reduced childbirth rates compared with ABVD in males, but not in females(32). Ideally, a larger study is needed to evaluate sperm quality after eBEACOPDac and a study with longer follow-up to analyse the birth rate after treatment.

In conclusion, our study provides strong supportive evidence that eBEACOPDac is highly efficacious Hodgkin lymphoma therapy. The clear benefits in terms of stem cell genomic health and fertility demonstrated here provide even more reason for clinicians offering frontline eBEACOPP to consider replacing procarbazine with dacarbazine. The prospective HD21 trial(28) has already demonstrated toxicity benefits of BrECADD compared with eBEACOPP and in healthcare environments with resources sufficient to fund Brentuximab vedotin, it is anticipated that BrECADD will become a widely adopted standard of care. However, in many less well-resourced healthcare environments replacing procarbazine with dacarbazine in frontline eBEACOPP is easily accomplished in routine clinical practice and can be implemented rapidly with minimal resource implications. This provides an immediate means to reduce treatment toxicity and improve genomic and reproductive health in young Hodgkin lymphoma patients across the world.

Contributors:

GAF, AKM and PJC conceived of the study. GAF, KS, EM and AS designed the study. AS, KS, PB, TFM, WO, TC, KMA, SB, KB, SB, NDC, GPC, DJC, KC, AD, AD, DD, MF, EGE, AH, DH, HH, SI, SGJ, MK, KML, OCL, NMC, AM, PM, SKN, EHP, NP, NKS, GS, AS, RT, BJU, NW, XYZ, AKM collected the clinical data from 25 centres. PB and SB contributed data from the HD18 clinical trial. AS, EM, RS, HLS and JB prepared the samples for mutational analysis in the laboratories of EL, RR and NC. EM, MHP, RS and HLS verified the data and performed the mutational analysis, supervised by RR, MRS, and PJC. AS, EM, MHP, JFR and JJ performed statistical analysis. The manuscript was first drafted by AS, EM, MHP, RS, RR and GAF. The manuscript was critically revised by GCP and SB. All authors gave final approval of the version to be published and have contributed to the manuscript.

Delcaration of interests:

AS reports support for meetings/conferences from Takeda. WO reports payment or honoraria for lectures, presentations, speakers, bureaus, manuscript writing or educational events from Roche, Takeda, Pfizer, Servier, Kite Gilead, MSD, Novartis, Beigene, Astra Zeneca, Syneos, Autolus, Kywoa Kirin, Abbvie, Incyte, BMS, Janssen, Sobi. GCP reports honoraria for speaker and advisory work from Takeda, Roche, Kite, Abbvie, Beigene, Astra Zeneca, Sobi, Secura Bio; and support for research from Beigene, Pfizer, BMS, Amgen, Astra Zeneca. DD reports speaker fees from Abbvie and support for travel to conferences from Astrazeneca and Beigene. PM reports support for meetings/conferences from Takeda. EHP reports honoraria for advisory work and support for meetings/conferences from Takeda. EL reports research support from CSL Behring. PB reports consulting fees from Takeda, BMS, Roche, Amgen, Novartis, Celgene, Miltenyi Biotech, and Gilead; payment or honoraria for lectures,

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Data sharing:

Data supporting this study are not publicly available due to confidentiality of the research participants. Please contact the corresponding authors (GAF and RR). The sample identifiers contained in this manuscript are not known to anyone outside of the research group.

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