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Original Research

# The role of genomic profiling in adolescents and young adults (AYAs) with advanced cancer participating in phase I clinical trials



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## KEYWORDS

Adolescent and young adult;  
AYA;  
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**Abstract** **Introduction:** Adolescents and young adults (AYAs) diagnosed with cancer between ages 15–39 years may harbour germline variants associated with cancer predisposition. Such variants represent putative therapeutic targets, as may somatic variants in the tumour. Germline and tumour molecular profiling is increasingly utilised to facilitate personalisation of cancer treatment in such individuals.

**Aim:** Considering AYAs with advanced solid tumours managed in a specialist drug development unit (DDU), the aims of this study were to investigate the use and impact of:

1. Germline genetic assessment.
2. Tumour molecular profiling.

**Methods:** AYAs treated in the DDU at the Royal Marsden Hospital between 2002 and 2016 were identified from departmental databases. Data regarding clinicopathological features, clinical assessments and germline and tumour genetic testing were retrieved by chart review.

**Results:** The study cohort included 219 AYAs. Common cancer types included sarcoma (41, 19%); cervical (27, 12%); breast (25, 11%); ovarian (23, 11%) and colorectal (21, 10%) cancers. Germline testing was undertaken in 34 (16%) patients, 22 of whom carried a pathogenic variant. Using current testing criteria, an additional 32 (15%) would be eligible for germline

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testing based on their personal history of cancer alone. Tumour testing was undertaken in 46 (21%) individuals. Somatic mutations were commonly identified in *TP53* 13 (28%); *PIK3CA* 8, 18%; *KRAS* 4, 9% and *MET* 5 (11%).

**Discussion:** A significant proportion of AYAs with advanced cancer have targetable somatic or germline mutations. Consideration of familial risk factors and inclusion of germline testing wherever appropriate can complement tumour testing to optimise patient management and inform management of at-risk relatives.

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## 1. Introduction

The aim of phase I clinical trials is to determine the safety and tolerability of experimental agents in humans [1]. Because of the unpredictable side effect profile and indeterminate efficacy of these agents, the majority of patients referred for consideration of such trials generally have advanced stage, heavily pre-treated tumours, or rare tumours with a paucity of available standard therapies. To fulfil eligibility criteria for such trials, patients must be of acceptable medical fitness (ECOG<sup>2</sup> 0 or 1) [2], such that they have enough physiological reserve to cope with unexpected side effects. The patient population of phase I units is therefore enriched for younger patients, despite the relative rarity of cancer in individuals under the age of 40. Other factors are also considered in determining eligibility for phase I trials, including previous therapies and current medications and comorbidities. Although the aim of the phase I trial is to determine safety of experimental agents, patients are preferentially allocated to trials with best predicted likelihood of efficacy based on preclinical evidence, given that patient outcomes are known to be better when therapy is targeted [3–5]. Molecular characterisation of tumours by sequencing or by immunohistochemistry may inform decision-making with respect to allocation to a trial of a targeted agent, immunotherapy or anti-angiogenic agent depending on the molecular defect identified [6,7]. As genomic technologies have improved and become more affordable [8], the methods by which this molecular characterisation can be routinely undertaken have changed dramatically in our institution, moving from hotspot mutational analysis by Polymerase Chain Reaction (PCR) to sequencing by next generation technologies using multi-gene panels. Our panels have also expanded over time to reflect increases in the size of the portfolio of targeted agents under investigation in our unit. The panel currently in use in our unit includes 113 ‘DNA damage repair’ genes, including tumour suppressor genes such as *BRCA1*, *BRCA2* or mismatch repair genes.

Individuals diagnosed with cancer between the ages of 15 and 39 are referred to as ‘adolescents and young

adults (AYAs)’ [9]. Cancers occurring in this cohort may include paediatric/teenage cancers, cancers of young adulthood, and adult cancers occurring at unexpectedly young ages [10–12]. Such phenomena are characteristic of inherited cancer predisposition syndromes. Certain cancer types [13] occurring in this age group are also strong predictors of an underlying germline defect. The approximate likelihood of identifying a pathogenic mutation in *TP53* in an AYA with an adrenocortical cancer, for example, is 13% [14], which is lower than the probability of identifying a mutation in a child with the same cancer (50–80%) [15–17], but over twice that of older adults (5.8%) [18]. The characteristic cancer occurring in individuals with *CDH1* mutations is diffuse gastric cancer, and the mean age of diagnosis in affected individuals is 38–40 years [19,20]. The association between high grade serous ovarian cancer and triple negative breast cancer with *BRCA1/BRCA2* mutations is well-established [21–23], and the likelihood of detecting a mutation in one of these genes in an affected individual is inversely proportional to the age at diagnosis. For this reason, in our institution, germline genetic testing is offered to individuals diagnosed with these and other certain types of cancer at age less than 40 years, irrespective of family history (Table 1). For other tumour types, tumour-based immunohistochemical analysis is recommended. In 2017, the NICE guidelines were updated to include a recommendation that all individuals diagnosed with colorectal cancer should have analysis of their tumour by IHC for mismatch repair proteins, or microsatellite stability testing, to guide further testing for Lynch syndrome [24]. In our institution, routine IHC is also performed on all endometrial cancers for the same purpose.

We conducted a study of AYA patients undergoing phase I clinical trials at the Drug Development Unit, Royal Marsden Hospital UK. One aim of this study was to investigate the proportion of individuals in this cohort with inherited cancer predisposition syndromes and to identify how many additional individuals in this cohort would be eligible for germline investigations using current testing criteria. A second aim of this study was to examine

<sup>2</sup> ECOG: Eastern Cooperative Oncology Group.

Table 1

Indications for genetic testing in our unit.

Cancer	Gene	Indication for testing
Breast	<i>BRCA1/2</i>	All patients <45y
	<i>TP53</i>	All patients <30 if <i>BRCA1/2</i> testing uninformative
Colorectal	MMR IHC ± germline analysis	All patients
	<i>APC/MUTYH</i>	CRC + ≥3 adenomatous polyposis
Sarcoma	<i>MUTYH</i>	CRC + 2 affected sibs
	<i>TP53</i>	Rhabdomyosarcoma <5y Other sarcomas, depending on family history
Adrenocortical cancer	<i>TP53</i>	All patients
Thyroid	<i>RET</i>	All medullary thyroid cancers
	<i>PTEN</i>	Depends on clinical features
Kidney	Renal panel	All patients <40y
Paraganglioma/pheochromocytoma	PGL/phaeochromocytoma panel	All patients
Ovarian	<i>BRCA1/2</i>	All epithelial ovarian cancer
Stomach	<i>CDH1</i>	All patients <45y
Endometrial	MMR IHC ± germline analysis	All patients
	<i>FH/PTEN</i>	Depends on clinical features

the utility of germline and somatic testing in determining trial allocation in this patient cohort.

## 2. Methods

### 2.1. Germline genetic assessment

In recent years, germline genetic testing at our institution has been performed using the Illumina TruSight cancer predisposition gene panel. After library preparation, sequencing is performed on the Illumina HiSeq2500. Although all genes on the panel are sequenced, only data pertaining to the genes of interest according to the clinical request form are analysed. Sequencing data are analysed using a bespoke pipeline. Rare and/or pathogenic variants are confirmed by Sanger sequencing or Multiplex Ligation-dependent Probe Amplification (MLPA) [25]. Testing in other units utilises similar next generation sequencing technology. Prior to the availability of next generation sequencing, sequencing of single genes was generally performed by Sanger Sequencing and MLPA. Currently, 90% of results from diagnostic *BRCA1* and *BRCA2* analysis are reported within 3 weeks, and 80% of results from diagnostic analysis of other genes within 4 weeks [26].

### 2.2. Somatic genetic assessment

Testing of tumours for potentially targetable mutations has been undertaken in our unit since 2011, commencing initially with testing of tumours for recurrent hotspot mutations in known driver genes, followed by sequencing of limited numbers of genes and moving more recently to next generation sequencing of tumours using multi-gene panels. Tumours from 28 patients were analysed using a 48-gene TruSeq® Amplicon Cancer Panel, and tumours from eleven patients were analysed using a custom-designed 113-gene ‘DNA damage repair’ panel. Tumours from 7 other patients were tested using multi-gene

panels, but reporting of results was limited uniformly to *ATM*, *BRAF*, *EGFR*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA* and *TP53* and variably to other genes of interest depending on the cancer type. In addition, select patients underwent immunohistochemical assessment of *PTEN* and *ATM*. For the purposes of this study, only results from multi-gene panel testing were analysed. The selection of patient to whom somatic testing was offered was largely dependent on portfolio of trials available within the unit at the time of the patient referral. Tumour molecular characterisation was offered to those patients for whom eligibility for a trial depended on presence/absence of particular molecular aberrations.

Compared to germline variants, where the American College of Medical Genetics has defined strict guidelines for determination of pathogenicity, there is a paucity of guidance with respect to classifying somatic variants. Sequencing data were analysed using a bespoke pipeline, and variants identified with frequency greater than 5% were reported. Variants were classified as ‘high’, ‘medium,’ or ‘low’ impact depending on type of variant and impact on protein and on evidence from Clinvar and COSMIC databases. All results were discussed at a multi-disciplinary meeting. Other factors in determining whether a variant might be considered a driver mutation included tumour type, frequency of the variant in the tumour relative to tumour content analysed and published literature regarding the variant in question. Data to support/refute pathogenicity of the same variant in the germline were also considered.

### 2.3. Phase I trial allocation

All patients referred for consideration of a phase I trials undergo initial clinical review to determine overall fitness to proceed based on current performance status, past medical history, medications and previous treatment. All cases are discussed at a weekly Patient Allocation Meeting, where this assessment and other factors, such as

tumour type, results of germline and somatic assessment and available trial slots are considered. Ideally, patients with confirmed pathogenic variants in the germline or soma are allocated to trials investigating utility of agents targeting the gene in question or a key pathway in which it is involved. In the case that no available trial exists, or if no slot is available on an existing trial, patients are allocated to the next best available trial depending on tumour type and patient factors. If rare variants of uncertain significance were identified in genes in which driver mutations were known to be associated with the tumour of interest, and if a trial using a targeted agent was available, cases were discussed with trial sponsors to determine patient eligibility.

### 3. Results

#### 3.1. Patient characteristics

Two hundred and nineteen patients diagnosed with cancer between the ages of 15 and 39 were referred to the Drug Development Unit in the Royal Marsden Hospital during the study period (2002–2016), including 139 (63%) females and 80 (37%) males. The median age of patients was 32 (15–39). The most common cancer type affecting these patients was sarcoma ( $n = 41$ , 19%, Table 2), of which the most common subtype was Ewing ( $n = 14$ , 34%, Table 3). Sarcoma was the most common cancer type in males (25, 31%), followed by colorectal ( $n = 13$ , 16%), while the most common cancers in females in this cohort were cervical ( $n = 27$ , 19%), breast ( $n = 25$ , 18%) and ovarian ( $n = 22$ , 16%, Table 2). Most patients had undergone at least one line of systemic therapy in the metastatic setting before consideration of a phase I clinical trial (Table 4), but the

Table 2

Cancer types in male and female AYAs.

Cancer type	N (%)		
	Female (n = 139)	Male (n = 80)	Total (n = 219)
Sarcoma	16 (12)	25 (31)	41 (19)
Cervix	27 (19)	0	27 (12)
Breast	25 (18)	0	25 (11)
Ovarian	22 (16)	0	22 (10)
Colorectal	8 (6)	13 (16)	21 (10)
Melanoma	10 (7)	7 (9)	17 (8)
Brain	6 (4)	8 (10)	14 (6)
Adrenocortical	5 (4)	2 (3)	7 (3)
Cholangiocarcinoma	3 (2)	4 (5)	7 (3)
Head and Neck	2 (1)	3 (4)	5 (2)
Renal	2 (1)	3 (4)	5 (2)
Germ cell	1 (1)	3 (4)	4 (2)
HCC	1 (1)	3 (4)	4 (2)
Lung	3 (2)	1 (1)	4 (2)
MCUP	1 (1)	3 (4)	4 (2)
Bladder	1 (1)	1 (1)	2 (1)
Endometrial	2 (1)	0	2 (1)
Others	4 (3)	4 (5)	8 (4)

Table 3  
Subtypes of sarcoma in male and female AYAs.

Subtype of Sarcoma	N (%)		
	Female	Male	All patients
Ewing sarcoma family	5 (31)	10 (40)	15 (36.6)
Malignant peripheral nerve sheath tumour	1 (6)	3 (12)	4 (9.8)
Osteosarcoma	1 (6)	3 (12)	4 (9.8)
Desmoplastic small round cell tumour	2 (13)	1 (4)	3 (7.3)
Alveolar soft part sarcoma	1 (6)	1 (4)	2 (4.9)
Chondrosarcoma	2 (13)	0	2 (4.9)
Synovial sarcoma	2 (13)	0	2 (4.9)
Clear cell sarcoma	0	2 (8)	2 (4.9)
Chordoma	0	1 (4)	1 (2.4)
Fibrosarcoma	0	1 (4)	1 (2.4)
Undifferentiated pleomorphic sarcoma	0	1 (4)	1 (2.4)
Pulmonary sarcoma	0	1 (4)	1 (2.4)
Rhabdomyosarcoma	0	1 (4)	1 (2.4)
Leiomyosarcoma	1 (6)	0	1 (2.4)
Spindle cell sarcoma	1 (6)	0	1 (2.4)
	16	25	41

majority had a relatively short interval between diagnosis and referral for consideration of a phase I agent (median time to referral 29 months [1–237], Table 4).

#### 3.2. Family history of cancer

In 81 (37%) individuals, no family history information was documented in either the patient's referral documents or clinical notes. Twenty-four patients were noted to have a positive family history including a first degree relative with cancer. In ten cases, the positive family history was of a cancer with a potentially shared genetic aetiology. Forty-two patients had at least one second-degree relative with cancer, 19 of whom had a cancer with potentially shared genetic susceptibility. The family history of 40 individuals in this cohort was deemed 'non-contributory' in the clinical notes, but no detail was given as to whether the information sought was limited to first degree relatives, or whether a history of other cancers was present in the family.

#### 3.3. Germline genetic assessment

Thirty-four patients (16%) were documented to have had germline genetic testing, of whom 22 were reported to have pathogenic variants (Table 5). Pathogenic variants were most commonly reported in *BRCA1* ( $n = 15$ ) or *BRCA2* ( $n = 3$ ), in 8 patients with ovarian cancer and ten patients with breast cancer (Table 6). In 12 cases, genetic reports were not available for review, as they had not been provided at the time of patient referral and were not actively requested.

One variant of uncertain significance in *BRCA2* (c.9205T>C; [p.Cys3069Arg]) was detected in a single patient diagnosed with hormone receptor and HER2-receptor-positive breast cancer at 23 years of age. It

Table 4

Previous treatments and time to referral for different cancer types in AYAs.

Cancer type	Median (Range)	
	Number of previous lines of treatment	Length of time between diagnosis and referral to DDU (months)
Sarcoma	2 (0–6)	26 (2–164)
Cervical	1 (0–3)	22 (6–60)
Breast	2 (1–5)	52 (11–128)
Ovarian	2.5 (0–6)	31.5 (6–237)
Colorectal	2 (1–4)	23 (11–78)
Melanoma	1 (0–3)	43 (1–110)
Brain	2 (0–3)	55 (8–136)
Adrenocortical	1 (1–2)	20 (8–52)
Cholangiocarcinoma	2 (1–3)	17 (6–56)
Head and neck	1 (1–3)	13 (10–32)
Renal	2 (0–2)	26 (17–47)
Germ cell tumour	2 (2–3)	99 (9–201)
Hepatocellular cancer	2 (1–4)	42.5 (5–151)
Lung	2.5 (2–3)	12.5 (11–21)
Bladder	1.5 (0–3)	19.5 (11–28)
Endometrial	2.5 (2–3)	21 (19–23)
Others	1 (1–5)	27.5 (13–70)
Metastatic cancer of unknown primary	2 (1–3)	11.5 (10–14)
All cases	2 (0–6)	29 (1–237)

was not documented that *TP53* analysis had been undertaken in this individual.

One patient with early-onset Triple Negative breast cancer had undergone predictive testing for a known

familial *BRCA2* mutation, in advance of her own diagnosis, but unfortunately was not referred for full diagnostic testing subsequently.

The majority of genetic testing was undertaken after formal consultation with a genetic specialist. Three patients had analysis of *BRCA1* and *BRCA2* as part of the mainstreaming genetic testing pathway [27,28]. Using current guidelines, an additional 18 patients (10 breast, 8 non-mucinous ovarian) would now qualify for mainstreamed *BRCA1/BRCA2* analysis in our institution. One of these individuals had been assessed by a clinical geneticist at the time of her diagnosis but did not fulfil the testing criteria at the time of her review and died before criteria for testing were loosened.

Two other patients with breast cancer were reported to have inherited cancer predisposition syndromes, one with Cowden syndrome and another with type I Neurofibromatosis (NF1). Two other individuals with NF1 were included in this cohort, both of whom were diagnosed with malignant peripheral nerve sheath tumours. Two patients with Familial Adenomatous Polyposis (FAP) were also referred for consideration of phase I trials, one of whom was diagnosed with colorectal cancer and the other with a desmoid tumour. One patient with Von Hippel Lindau syndrome was assigned to a phase I trial after developing metastatic clear cell cancer of the kidney. Uninformative germline testing of *RET* was undertaken in 1 patient with medullary thyroid cancer. *MUTYH* analysis was undertaken in 1

Table 5

Germline genetic testing in AYAs.

Cancer Type	N	Germline genetic assessment undertaken	Positive result/clinical diagnosis	Eligible for germline genetic testing based on personal history only using current guidelines
Sarcoma	41	0	2	0
Cervix	27	0	0	0
Breast	25	14	12	12
Ovarian	22	13	8	8
Colorectal	21	2	1	0 <sup>a</sup>
Melanoma	17	0	0	0
Brain	14	0	0	0
Adrenocortical	7	0	0	7
Cholangiocarcinoma	7	0	0	0
Head and neck	5	1	0	0
Renal	5	1	1	5
Germ cell	4	0	0	0
Hepatocellular carcinoma	4	0	0	0
Lung	4	0	0	0
Metastatic cancer of unknown primary	4	0	0	0
Bladder	2	0	0	0
Endometrial	2	1	0	0 <sup>a</sup>
Others	8	2	1	0
Total	219	34 (16%)	25 (11%)	32 (15%)
	N	IHC analysis performed	Loss of ≥1 MMR protein	Additional patients eligible for IHC analysis
Colorectal	20	3 (15%)	0	17 (85%)
Endometrial	2	1 (50%)	0	1 (50%)

<sup>a</sup> Unless directed by IHC analysis.

Table 6

Germline variants identified in AYAs.

Gene	Pathogenic variant		Number of patients	Cancer in patient carrying variant
<i>BRCA1</i>	c.427G>T	p.Glu143Ter	1	Breast
<i>BRCA1</i>	c.1505_1509delTAAAG	p.Leu502Serfs	1	Ovarian
<i>BRCA1</i>	c.3331_3334delCAAG	p.Gln1111fs	2	Breast (1) Ovarian (1)
<i>BRCA1</i>	c.3756_3759delGTCT	p.Ser1253Argfs	1	Ovarian
<i>BRCA1</i>	c.4327C>T	p.Arg1443Ter	1	Breast
<i>BRCA1</i>	c.4574_4575delAA	p.Gln1525Argfs	1	Ovarian
<i>BRCA1</i>	c.5266dupC_p.Gln1756ProfsX74)	p.Gln1756Profs	1	Breast
<i>BRCA1</i>	c.5278-1G>T	p.?	1	Breast
<i>BRCA1</i>	Duplication exon 13	p.?	1	Breast
<i>PTEN</i>	Unknown, no report available for review		1	Breast
<i>APC</i>	Unknown, no report available for review		2	Colorectal (1) Desmoid (1)
<i>VHL</i>	Unknown, no report available for review		1	Kidney
<i>BRCA1</i>	Unknown, no report available for review		5	Ovarian (4) Breast (1)
<i>BRCA2</i>	Unknown, no report available for review		3	Breast (3)

patient with early-onset mismatch repair-proficient colorectal cancer. Uninformative results were obtained from a multi-gene panel including *CDKN1B*, *VHL*, *NFI* and *MEN1* in a patient with thymic neuroendocrine tumour. One patient with early-onset endometrial cancer underwent formal genetic assessment, tumour immunohistochemical assessment of mismatch repair proteins (MMR IHC) and germline *FH* (*Fumarate Hydratase*) testing. No underlying genetic defect was identified. MMR IHC was also performed on the tumours of two patients with early-onset colorectal cancer, which was normal in both cases. No further genetic assessment was undertaken in these individuals. Using current guidelines [29], the 17 other patients in this series with colorectal cancer that did not have any such investigations performed should have, at least, MMR IHC or microsatellite instability of their tumours to out-rule Lynch syndrome. In our centre, the other young patient with endometrial cancer would also have MMR IHC analysis under contemporary institutional practice (Table 5).

Three patients in this series had more than 1 primary cancer, including one individual with adrenocortical cancer aged 34 and secondary AML, one patient with breast cancer at 37 and previous history of Wilms' tumour at the age of 12; neither of whom were referred for clinical genetics assessment, and one patient with metastatic olfactory aesthesioneuroblastoma with past medical history of Hodgkin's lymphoma who was referred for genetics consultation, but died while awaiting an appointment.

#### 3.4. Somatic genetic assessment

In thirteen tumours tested using the 48-gene panel, no somatic variants with allele frequency greater than 5% (*major variants*) were identified. At least one major variant (at least 5%) was identified in 26 tumours (58%). Genes in which mutations were most commonly identified included *TP53*, *KRAS*, *PIK3CA* and *MET* (Fig. 1).

Increasing the panel size to 113 genes increased the yield of variants, but the majority of variants identified in genes that were not included on the 48-gene panel were either benign/passenger mutations, or pathogenic variants for which no targeted therapy currently exists.

#### 3.5. Use of genomic profiling in trial allocation

Of 18 patients with pathogenic *BRCA1/BRCA2* variants, 13 (72%) were allocated to a trial investigating utility of a Poly-ADP-ribose polymerase (PARP) inhibitor. Four other BRCA mutation carriers were allocated to trials investigating an agent involved in dsDNA damage response pathways. Considering all individuals ( $n = 46$ ) undergoing tumour molecular characterisation, 9 were allocated to trials because of their underlying germline defect and 7 assigned to trials of agents targeting identified somatic mutations or genes in a related pathway. Potentially actionable somatic mutations were identified in 15 individuals, but at the time of their referral to the unit, there was either no suitable trial or no slots available on a trial of interest. In 3 patients, allocation to a trial was not altered by tumour profiling, and assignation was on their tumour histology (Ewing sarcoma,  $n = 2$ ) or immunophenotype (HER2-positive breast cancer,  $n = 1$ ). In 3 patients, the crude number of mutations identified was considered a surrogate marker of high mutational load; and they were therefore assigned to trials of immunotherapy. For the other patients, no informative data were derived from germline or somatic genomic investigations.

## 4. Discussion

Cancer treatment in AYAs poses a unique set of challenges. Tumours in this cohort of individuals have been shown to demonstrate distinct biological behaviours compared to tumours of the same age occurring in the

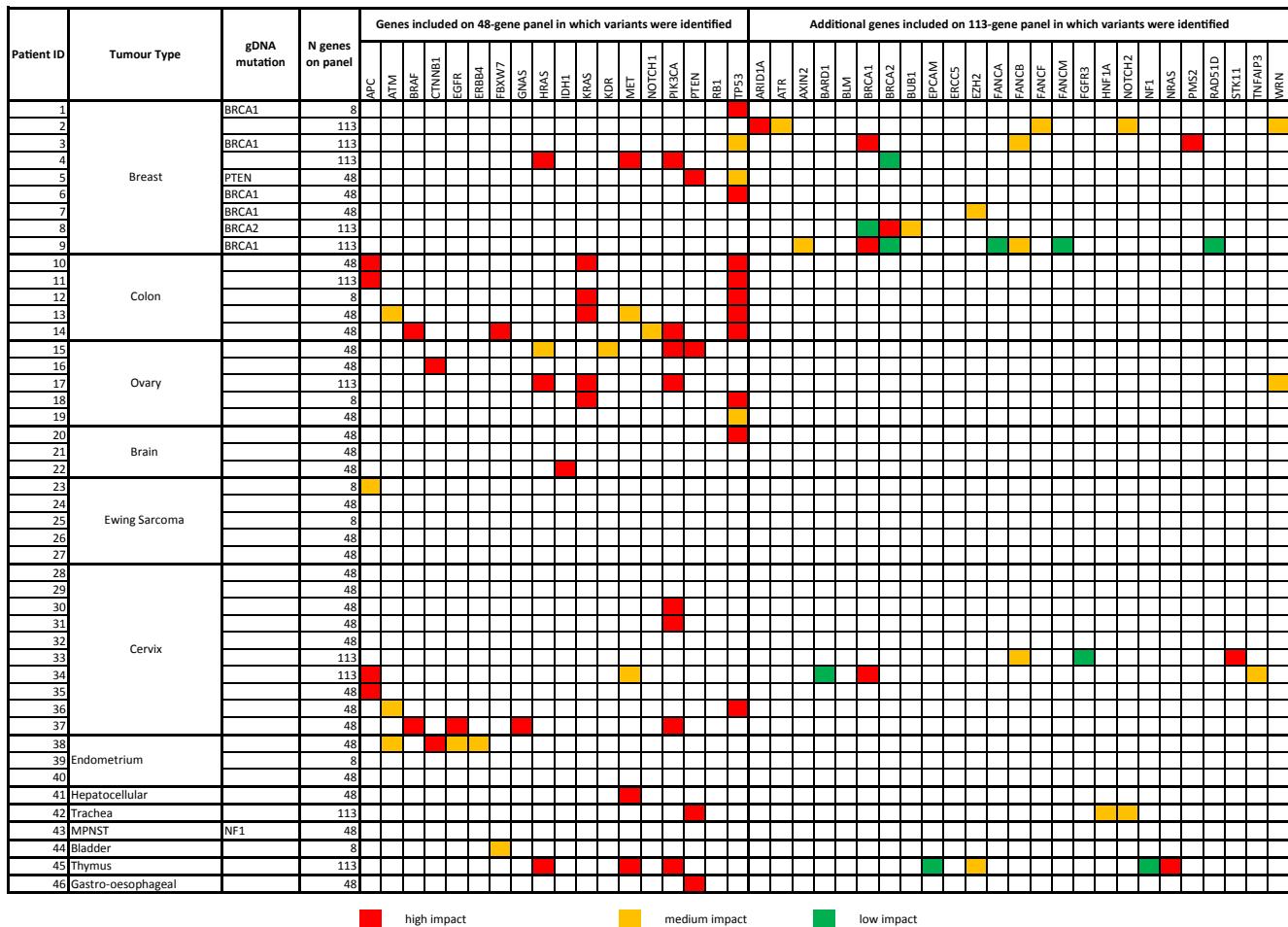


Fig. 1. Variants identified in tumour DNA.

paediatric or older adult setting [9,30–32]. Furthermore, this cohort of individuals has unique psychosocial needs and may have parents, siblings or young children for whom a familial risk of cancer may be relevant.

Recognition of a heritable cause of cancer is essential to provide optimal care to individuals with cancer. Identification of germline defects may modify therapeutic decisions [33]; for example, germline mutations in *BRCA1* or *BRCA2* tend to confer sensitivity to platinum-based chemotherapy [23], while identification of a *TP53* germline mutation may alter surgical decision-making, or influence radiotherapy use or dose [34]. In line with recent NICE guidelines [24], assessment of mismatch repair proteins by IHC is performed routinely in our institution on all colorectal cancers; and more recently, is also being routinely performed on all endometrial cancers. Identification of microsatellite instability or mismatch repair deficiency may help direct application of 5-FU based chemotherapy in high-risk Stage II (Duke's B) colon cancer [35] and may also indicate the presence of a germline mutation causing Lynch syndrome.

In this cohort of unselected AYAs attending a phase I unit, investigations to confirm/out-rule an inherited

cancer predisposition syndrome was, or would be undertaken using current guidelines in 89 (41%) patients, based on their personal cancer history alone. In this cohort, usually only a limited family history was recorded. In 81 (37%), no family history information was documented in any of the patient's referral documents or clinical notes, and in an additional 40 patients, the family history was deemed 'non-contributory' but may not have expressly included a 3-generation history. It is likely therefore that genetic investigations would have been indicated in an even greater proportion if a detailed family history was taken into account.

In this particular cohort, the affected individuals had advanced cancer with poor prognoses. However, in AYAs with earlier stage cancer, the risk of second malignancies associated with an inherited cancer predisposition syndrome is increased, and identification of a germline defect presents an opportunity to instigate prophylactic interventions. Furthermore, the significant risk to siblings, parents, and children of individuals with inherited cancer predisposition syndromes should also be considered and preferably be discussed at an earlier stage of the disease. Consideration of a possible inherited germline defect in an individual in the palliative or

experimental trial setting may provide the last opportunity for investigation in a family. In situations where an affected proband dies prior to genetic investigation, germline testing in the family is often impossible, as a negative result in an unaffected relative may not be reassuring to other individuals in the family. The results of this study suggest that germline genetic investigations should be routinely considered in AYAs with cancer, as the result may have a direct impact on management or allocation to a clinical trial.

Previous studies have shown that tumour-based genetic testing can inadvertently unmask a germline mutation in 3–5% cases [36,37]. In our cohort, pathogenic variants were identified in *BRCA1* and *BRCA2* in tumours of two individuals with breast cancer. These results were not unexpected, as in both cases, the patients had previously been known to carry germline mutations. It is likely that, as more tumour testing is undertaken using the 113-gene panel, more variants in cancer susceptibility genes will be identified. It is important, therefore, to bear the possibility of unmasking a germline event in mind, particularly if the patient is young, or with a positive family history suggesting an inherited cancer predisposition. At the present time, germline analysis to confirm constitutional status of a mutation in a particular gene in a patient without a positive family history may not be funded by the national health system (NHS), particularly if the mutation was identified in a tumour not known to be associated with germline mutations in that gene. To avoid this scenario, some units use germline DNA subtraction when interpreting their tumour sequencing results. This approach has the disadvantage of potentially missing targetable mutations. In our unit, and in most other oncology units, germline testing for the purpose of matched analysis is not routinely performed. All tumour results are discussed at a departmental clinical meeting. If any variant is suspected to be germline in origin, the case is discussed with the cancer genetics service, and germline testing undertaken, if appropriate. Due attention must be paid to the slight possibility of incidental results of this nature when consenting individuals for such testing, and concerns about how consent for such testing should be undertaken have been discussed within our unit and indeed internationally.

Interpretation and classification of somatic variants is extremely challenging. Where classification of germline variants is guided by stringent guidelines such as those of the American College of Medical Genetics (ACMG) [38] and Association for Clinical Genetic Science (ACGS) [39], no robust classification system exists for somatic variants. In tumours, pathogenicity is not an immediate indicator that the mutation is driving the neoplastic process. This must be considered when a variant is identified in a tumour in a gene in which mutations have not previously been reported to drive the specific cancer. Clinical utility of the test is an

important factor to consider before offering the test, as identification of variants of uncertain significance, or variants in genes of uncertain significance may not be informative, but may generate significant anxiety and additional clinical work. Interpretation of somatic variation is further complicated by mutation frequency—a suspected driver mutation with low mutation frequency may represent a driver mutation in only a subclonal population of cells or may be a late passenger event. Tumour heterogeneity adds complexity to interpretation of tumour testing, and factors such as site of the biopsy (central or peripheral; primary or metastasis), previous treatments and potential secondary mutational events should be considered.

Twelve patients were reported to have been identified as germline mutation carriers, but genetic reports had not been formally requested by our institution. We strongly recommend that genetic reports formally be reviewed, to confirm that the variant identified was pathogenic/likely pathogenic, and therefore clinically actionable. Genetic testing for NF1 is not routinely advocated, as this condition can be diagnosed on a clinical basis, and genetic testing is often uninformative if cDNA analysis has not been undertaken [40,41].

The relative frequency of specific cancer types in our cohort was biased by availability of different trials over the study trial; the cohort was enriched for Ewing sarcoma because of an ongoing IGF1-inhibitor trial; and for breast and ovarian cancers because of PARP-inhibitor studies.

## 5. Conclusion

Overall, 34 AYA patients (16%) did have germline genetic assessment, 3 (1%) had a confirmed genetic diagnosis and 2 (1%) had tumour-based investigations. Using current testing criteria, an additional 32 (15%) of patients would qualify for a germline genetic test based on their personal history, and another 18 (8%) would have MMR IHC of their tumour. It is critical that heritable cancer predisposition be recognised in AYA patients in general, as it may have significant implications for the treatment and surveillance of the proband and his/her relatives. Ideally, diagnosis of a cancer predisposition syndrome should occur at an early stage in the patient's treatment, to guide decision-making. The phase I setting is not an ideal scenario in which to discuss genetic testing and implications for the family. In this setting, patients will be discussing clinical trials and tumour molecular profiling, and discussing germline testing as well may be confusing or overwhelming. The potential impact to the wider family may also cause psychological distress in a vulnerable individual dealing with a life-limiting illness with little therapeutic options. Conversely, the phase I setting may provide one of the last opportunities to offer germline testing to these

patients, and by extension, their families. Overall, 40% of this cohort would have qualified for germline genetic testing using today's criteria based on their personal history alone, and it is likely that a greater proportion still would also qualify if accurate family history information was recorded. Involvement of a cancer geneticist routinely in the multidisciplinary care of this unique young cohort would help optimise the overall treatment of the patient and their family, particularly in cases where the proband has limited life expectancy.

### Conflict of interest statement

None declared.

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