A phase I trial of the PARP inhibitor olaparib in patients with relapsed or refractory Chronic Lymphocytic Leukaemia, T-Prolymphocytic Leukaemia or Mantle Cell Lymphoma.

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Abstract

Relapsed chronic lymphocytic leukaemia (CLL), mantle cell lymphoma (MCL) and T-prolymphocytic leukaemia (T-PLL) remain incurable with current chemotherapy. Mutations in genes involved in DNA damage response (DDR) are common in these disorders and may represent an opportunity to exploit Poly (ADP-ribose) polymerase (PARP) inhibitors that promote synthetic lethality and have shown marked efficacy in the treatment of solid tumours with homologous recombination repair (HRR) defects.

We performed a phase I trial to determine the maximum tolerated dose (MTD) and safety of the PARP inhibitor olaparib in 15 patients with relapsed CLL, MCL or T-PLL. The MTD of the capsule formulation was defined as 200mg twice daily (bd). The tablet formulation was tolerated at 100mg bd but the MTD was not defined. Myelosuppression was the main toxicity with grade ≥3 AEs observed in 66% of patients (grade ≥3 anaemia (5 Patients; 33%), thrombocytopenia (5 patients; 33%) and neutropenia (3 patients; 20%). The median survival of patients within the trial was 129 days. There appeared to be a trend towards longer treatment duration and increased survival in patients with DDR gene mutations. Olaparib may represent a useful therapy for patients with lymphoid tumours harbouring DDR alterations and further studies are warranted.

Introduction

Despite the array of therapeutic options in patients with chronic lymphocytic leukaemia (CLL), mantle cell lymphoma (MCL) and T-prolymphocytic leukaemia (T-PLL), chemoresistance remains a major problem, particularly in advanced disease. Alkylating agents and purine analogues are the traditional chemotherapy agents used in the management of these malignancies and induce apoptosis in target cells through an *ATM-p53* DNA damage response (DDR)-dependent pathway. As such, genetic alterations in the *ATM-p53* DDR pathway represent an important mechanism of chemoresistance and it has been shown that defects and functional loss of DDR genes are associated with poor prognosis (Austen*, et al* 2005, Austen*, et al* 2007, Skowronska*, et al* 2012, Zenz*, et al* 2010, Zenz*, et al* 2011).

In recent years, several agents that act independently of the *ATM-p53* DDR pathway have become available, including immunotherapies and signalling inhibitors. These have markedly improved the clinical outcome for patients with CLL (Ghia and Hallek 2014, Hallek 2013, Hallek*, et al* 2010) although *ATM* or *TP53* gene abnormalities still impact on treatment response due to associated genomic instability which drives clonal evolution and promotes the selection of resistant (Byrd*, et al* 2013, Ouillette*, et al* 2010, Salin*, et al* 2008, Woyach*, et al* 2014). As such, there remains a need to develop new agents for the treatment of aggressive lymphoid tumours with DDR inactivation.

The ataxia telangiectasia-mutated (ATM) protein plays a critical role in the DNA damage response to double strand breaks (DSBs) (Shiloh and Ziv 2013) and

phosphorylates multiple proteins to facilitate either cell cycle arrest and DNA repair or apoptosis. Loss of ATM function in lymphoid tissues leads to the propagation of cells which harbour erroneously resolved DSBs that are associated with increased risk of malignant transformation. Indeed, individuals with inherited mutations in both *ATM* alleles have a highly increased risk of developing a wide range of B- and T-cell lymphoid malignancies (Stankovic*, et al* 1998). Somatic *ATM* mutations have also been found in a range of sporadic lymphoid tumours including B-CLL, MCL and T-PLL (Gronbaek*, et al* 2002, Stilgenbauer*, et al* 1997, Vorechovsky*, et al* 1997). These may occur in the form of monoallelic intra-chromosomal deletions of 11q or as biallelic inactivation through the combination of 11q deletion and mutation of the remaining *ATM* allele.

One emerging strategy to target cells in which ATM function has been lost is the approach of 'synthetic lethality', which exploits the fact that when tumour cells are impaired in one DSB repair mechanism they become critically dependent upon alternative pathways (Bouwman and Jonkers 2012, Shaheen*, et al* 2011). Poly (ADP-ribose) polymerase (PARP) plays a central role in single strand break (SSB) repair and when the activity of this enzyme is inhibited unrepaired SSB lesions are converted into DSBs during DNA replication. The resolution of these DSBs subsequently requires activation of homologous recombination repair (HRR) proteins, such as BRCA or ATM. PARP inhibitors have therefore been used to target tumour cells that carry mutations in genes such as *BRCA* or *ATM* and demonstrate an HRR-deficient phenotype. In relation to haemopoietic tumours, we have previously demonstrated the utility of PARP inhibition as a targeted therapy for *ATM*-

defective CLL and MCL, both *in vitro* and in *in vivo* pre-clinical xenograft models (Weston*, et al* 2010).

Olaparib is an oral PARP inhibitor that has been used widely in patients with solid tumours such as *BRCA1/2*-mutated ovarian, prostate and gastric cancers (Bryant*, et al* 2005, Kaufman*, et al* 2015, Mateo*, et al* 2015). It is well tolerated and demonstrates significant activity both as a single agent and in combination with chemotherapy (Bang*, et al* 2015, Bendell*, et al* 2015, van der Noll*, et al* 2015). In contrast to solid tumours, there is limited data for PARP inhibitors in haematological malignancies. Mild myelosuppression has been observed during the use of olaparib in patients with solid tumours, particularly in combination with chemotherapy (Bang*, et al* 2015, Bendell*, et al* 2015, van der Noll*, et al* 2015). Myelosuppression may therefore act as a potentially limiting factor in the treatment of haematological malignancies and an early phase trial in such malignancies is required to assess safety and the maximum dose that can be tolerated.

Here we report the results of a phase I trial addressing the safety of the PARP-inhibitor olaparib in patients with relapsed CLL, T-PLL or MCL. Particular focus was on the significance of *ATM* mutation status in the response to therapy.

Materials and Methods

Patient population, trial design, treatment and outcome measures

The Parp Inhibitor in relapsed Chronic Lymphocytic Leukaemia (PICCLe) trial was designed as a single arm, multi-centre, open label phase I/II trial although the phase II trial did not commence. Eligible patients had relapsed CLL, MCL or T-PLL and were not considered to be appropriate for further conventional treatment. Importantly for the phase I trial, patients lacking a confirmed chromosome 11q deletion or an *ATM* mutation were not excluded. Further inclusion criteria involved an ECOG performance status of ≤ 2, and an estimated life expectancy of more than 16 weeks. Important exclusion criteria incorporated persisting (>8 weeks) severe pancytopenia caused by previous therapy rather than disease (neutrophils $<0.5 \times 10^9$/L or platelets $<50 \times 10^9$/L) and concomitant treatment with strong CYP34A inhibitors. Full eligibility criteria are given in the supplementary material (Figure S1). The clinical trial was approved by the UK National Research Ethics Service (NRES) Committee West Midlands - Solihull and performed in accordance with local ethical guidelines. Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Phase I trial was designed as a conventional dose escalation trial (cumulative 3+3 design) the primary objective being the assessment of safety and maximum tolerated dose (MTD) (Figure 1). Successive cohorts of patients (3 patients per cohort) were treated with a fixed dose of olaparib. The MTD was defined as the highest dose with observed dose-limiting toxicities, (DLTs) of 0 or 1 out of 6 patients, where the next higher dose has at least 2 observed DLTs out of up to 6 patients. The initial 3

cohorts (9 patients) received the original capsule formulation of olaparib. However, during the trial AstraZeneca developed a tablet formulation to improve drug loading and bioavailability and reduce the number of tablets required to achieve the desired dose. Consequently, a further 2 cohorts received the new tablet formulation (6 patients). A starting dose of 200 mg bd in capsule formulation and 100 mg bd for tablet formulation was specified in the protocol and was intended to be given until progression (Figure 1).

Safety and tolerability of olaparib was assessed using the Common Terminology Criteria for Adverse Events Version 4.0) (CTCAE v4.0). The number of patients experiencing toxicities is reported by dose level, category and grade of severity (Table 3). The DLT assessment period spanned 8 weeks from treatment initiation. Only patients that completed 8 weeks of treatment were evaluable, unless they experienced a DLT which was considered as an event. A DLT was defined as any drug related non-haematological toxicity of: grade ≥3, with the exclusion of nausea, vomiting and diarrhoea unless not ameliorated by symptom directed therapy, or grade 4 neutropenia lasting ≥7 days or grade 4 thrombocytopenia with platelets $<10x10^9$/L despite ≥7 days transfusion support. Patients whose DLT outcome could not be assessed due to death-caused withdrawal or disease progression during the 8 week period were replaced.

Molecular analysis

Specific primers for targeted deep sequencing of *ATM* (exons 4-65), *SF3B1* (exons 13-16), *TP53* (exons 4-10), *BIRC3* (exons 2-9), and *MyD88* (exon 5) were designed with the D3 Assay Design web-based tool (https://www.fluidigm.com/assays) (Table

SI). The Access-Array system (Fluidigm) was used to generate amplicon libraries from 15-50 ng genomic DNA, for each sample extracted using the QIAamp DNA blood mini kit (Qiagen, Manchester, UK). PCR products were barcoded, pooled, purified using Ampure XP beads (Beckman Coulter, High Wycombe, UK), quantified by 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) then paired-end sequenced using a 500-cycle kit (2x250) on the MiSeq platform (Illumina, Cambridge, UK). Typically, several thousand reads were captured per amplicon. Generated files were quality filtered by Trimmomatic (v0.3), retaining reads with a Q-score >30, in a 4 base sliding window followed by primer removal using cutadapt (v1.2.1). Resultant sequencing reads were aligned with the Human Reference Genome (hg19, GRCh37) using the Burrows-Wheeler Aligner-MEM algorithm (BWA-MEM, v0.7.5). Coverage was analysed using the Bedtools (v2.17) API and cstom Python script. Appropriate read group information was added by Picard (v1.128) and realigned with the Genome Analysis Toolkit (GATK, v2.7.1) IndelRealigner tool. Variant calling was performed by UnifiedGenoTyper (GATK), Pindel (v0.2.5a1) and Platypus (v0.7.9). Resultant files were annotated using Annovar (July 2013 release), results merged and inter-run frequency calculated by a custom Python script58. A selected number of mutations were validated either by Sanger sequencing, if allelic frequency exceeded 20%, or allele specific PCR if below 20%. *NOTCH1* (exon 34) analysis was performed by Sanger sequencing with an annealing temperature of 60°C using primers designed with Primer3 via NCBI Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Sayers*, et al* 2012) (Table SI)

Statistical analysis

No formal statistical analysis was performed. Data were summarised using descriptive statistics (number of patients, median, minimum and maximum) for continuous variables and frequency and percentage for discrete variables. Data are presented for all patients who started treatment.

Efficacy analyses on Overall Survival (OS) were not originally planned for the Phase I part of the trial, but these had been summarized as the trial did not proceed into Phase II. OS was defined in days from the start of treatment to the date of death as a result of any cause. Patients without a documented date of death will be censored at the last date a patient is known to be alive or lost to follow-up. Median survival time was calculated using Kaplan–Meier methodology (where appropriate). This was reported as a whole group, by formulation and by mutation status along with treatment duration which was also reported by mutation status. All analyses were performed using R software (Team 2015). However, as this was a phase I trial with a heterogeneous patient population in terms of diseases, previous lines of therapy, co-morbidities and variable doses of olaparib, the efficacy analyses were only preliminary and not to make conclusions about efficacy.

Results

1. Patient population, safety and tolerability, maximum tolerated dose and survival outcome

Patient population

A total of 15 patients with relapsed CLL (n=9), MCL (n=4) or T-PLL (n=2) were enrolled and evaluated (Table 1). The median age of patients was 69 years (range 53–77) with male/female ratio of 2/13. The median number of previous lines of therapy was 3 (range 1-7).

Safety and Tolerability

The median duration of olaparib treatment was 71 days with an interquartile range of 26 – 93 days. Myelosuppression was the most common haematological grade 3-4 toxicity and was seen in 8 patients. However as many of the patients had low white blood counts due to pre-existing disease the contribution of olaparib to the observed myelosuppression was not entirely clear for all patients. Of the 6 patients dosed at 200mg bd (capsule) 3 patients experienced grade ≥3 AEs, all 3 patients who were dosed at 400mg bd (capsule) experienced at least 1 grade ≥3 AE. For the tablet formulation of olaparib, from those 6 patients dosed at 100mg bd 4 patients experienced grade ≥3 AEs (Table 3).

Overall, both formulations of olaparib were generally well tolerated in our trial of high risk patients. The most common adverse events (AEs) were anaemia (66%), followed by decreased platelet count (53%), fatigue (53%), nausea (33%) and decreased neutrophil count (33%) (Table 3). Grade ≥3 AEs were reported for 10 patients (66%) with the most common of these again being anaemia and decreased platelet count in 5 patients (33%) each, followed by decreased neutrophil count in 3 patients (20%) (Table 3).

Maximum Tolerated Dose

The second primary objective of the Phase I component was to define the MTD of olaparib in this patient group. The original capsule formulation of olaparib was changed to an oral tablet during the trial and the MTD was therefore assessed separately for each of these formulations (Figure 1).

Six patients received olaparib capsules at the starting dose of 200mg bd and only one patient developed a DLT (Figure 1, Table 2). This DLT was due to development of grade 4 thrombocytopenia within 2 weeks of treatment initiation and occurred in an MCL patient with bone marrow involvement, who was heavily pre-treated and experienced relapse from an allogeneic stem cell transplant. Three patients went on to receive the higher dose of 400mg capsules bd and all developed DLTs which were possibly attributable to olaparib within 6 weeks of treatment initiation (Figure 1, Table 2). These DLTs were evident as grade ≥3 maculo-papular rash, grade ≥3 anorexia or weight loss and a grade 4 thrombocytopenia, in a patient who had pre-existing thrombocytopenia. The MTD for olaparib capsules was therefore defined as 200mg capsules bd.

The tablet formulation of olaparib was introduced at a treatment dose of 100mg bd and was administered to 6 patients (Figure 1, Table 2). One patient developed a fatal DLT which presented as an infective episode, renal failure (acute kidney injury) and bleeding with a high International Normalised Ratio (INR) on warfarin (Figure 1, Table 2). A further patient failed to complete the course due to disease progression. Unfortunately, at this point recruitment ceased and we were therefore unable to define an MTD for the tablet formulation.

Overall Survival

Nine deaths were observed during the trial period. The median OS from the start of treatment for all 15 patients was 129 days (Figure 2a). The median OS for patients treated with capsules (106 days) was not dissimilar to that for patients treated with tablets (129 days) (Figure 2b).

2. Molecular characteristics of tumours

Peripheral blood mononuclear cells were obtained prior to olaparib treatment from all 15 patients enrolled in this trial. These were subjected to deep sequencing of 6 well-established CLL 'driver' genes*: ATM, TP53, BIRC3, SF3B1*, *NOTCH1* and *MyD88.* Twelve patients (80%) had evidence of a mutation in at least one of the analysed genes (Figure 3a, Table S2). A further patient, TNO13, presented with monoallelic *ATM* loss due to an 11q deletion (Figure 3a, Table 2). It has recently been reported that tumours that carry *SF3B1* gene alterations have similar functional consequences to that of ATM loss, indicating that SF3B1 is itself involved in the DNA damage response pathway (Te Raa*, et al* 2015). This justified our strategy to observe *ATM* and *SF3B1* mutant tumours as a single group with defective DDR. Of note, mutations in either *ATM* or *SF3B1* were seen in 9 patients (60%; Figure 3a, Table 2). Patient TNO4 exhibited bialleic ATM loss by virtue of the co-presence of the UK founder ATM mutation c.7271T>G with an 11q deletion, both present at >90% frequency. Interestingly this patient also harboured a *NOTCH1* mutation, suggesting co-operation of two strong driver mutations in disease progression (Figure 3a,).

To assess how tumour mutation status impacted on the clinical outcome of patients in the trial we initially assessed genotype in relation to ‘time on treatment’. Treatment duration ranged from 8 to 133 days with a median of 83 days in patients whose tumours carried mutated DDR genes (within *ATM or SF3B1*; ‘mutated DDR’) compared to 37.5 days in those lacking such alterations (‘unmutated DDR’) (Figure 3b). A longer median survival time of 192 days was also seen in patients with ‘mutated DDR’ genotype compared to 89 days in the ‘unmutated DDR’ group (Figure 3c). Our preliminary observations, in a small cohort (n=15), suggest that aberrations in the ATM pathway may confer longer treatment duration and overall survival even among heavily pre-treated and relapsed CLL patients though.

Discussion

Olaparib has demonstrated clinical efficacy in many studies and is an approved indication for the treatment of patients with deleterious or suspected germline BRCA-mutated advanced ovarian cancer. Tolerability has generally been good and the main toxicities seen in patients with solid tumours have been low grade GI toxicity (nausea, vomiting), fatigue and anaemia. However, combining olaparib with other myelotoxic drugs has shown myelosuppression in patients with solid tumours (Bang*, et al* 2015, Bendell*, et al* 2015, van der Noll*, et al* 2015) and emphasises the need to define the optimal dose in patients with haematological tumours as a single agent or in any potential combination.

The phase I trial described here tested olaparib as a single agent in a heavily pre-treated group of patients with lymphoid tumours and defined the maximum tolerated dose as 200mg capsules taken bd. In contrast to solid tumours where use of a single agent 400mg bd capsule formulation is generally well tolerated the same dose led to 3 types of DLT (rash, thrombocytopenia, anorexia) in patients with haematological malignancies.

Although olaparib was initially available as a capsule, the tablet formulation of 300mg bd is now used in most studies. This change in formulation, which occurred during the trial, limited the number of patients who could be recruited to the trial but the findings still provide important preliminary information. We observed that, similarly to its use in the treatment of solid tumours, olaparib was generally well tolerated in patients with lymphoid malignancies but was associated with the development of myelosuppression in patients with an extensive history of pre-treatment or bone marrow involvement. However, it is important to note that many of the patients enrolled on the trial had very advanced disease following multiple lines of therapy. It was therefore difficult to establish with confidence whether toxicity was related to olaparib or disease progression and this reflects the challenge of testing novel drugs in patients who have received multiple lines of therapy. The trial also highlighted a number of major challenges associated with early phase clinical trials. Firstly, the unforeseen change from capsule to tablet formulation of olaparib led to a major suspension of recruitment for almost a year (351 days). Even though the MTD of olaparib was obtained for the capsule formulation after 9 evaluable patients, it was no longer relevant due to the very different pharmacokinetic properties of capsule and tablet formulation and competing trials prevented us from completing

recruitment. The latter factors were unavoidable although in retrospect the trial design was also a factor that impacted on slow recruitment. The conventional '3+3' recruitment design suffered from the requirement that all 3 patients in a cohort had to complete 8 weeks on the trial in order to assess the occurrence of DLT. In addition, time had to be factored in for safety data to be gathered and reviewed by the safety review committee before opening of the next cohort at the next dose. Hence there was an average suspension of recruitment between cohorts of up to 11 weeks and in retrospect, given a very good initial recruitment, a more efficient model-based design such as a modified time-to-event Continual Reassessment Method (Cheung 2011, Cheung and Chappell 2000) could have allowed for more flexible cohort sizes as well as shorter suspension periods between cohorts whilst ensuring safety. This greater efficiency in recruitment would have meant that the trial could have been completed in a shorter period of time. In recent years there has been increasing interest in the use of such efficient model-based designs as they offer the potential to treat more patients at optimal doses, reduce the number of patients who receive sub-therapeutic doses and provide greater accuracy in attaining the MTD (Mussai*, et al* 2014). However these designs are more resource intensive and require specialist software that was not available when this trial was first planned in 2008 (Jaki 2013).

The greatest efficacy of PARP inhibitors has been seen in tumours which carry genetic defects in homologous recombination and as such we examined tumour material for the presence of mutations within several CLL 'driver' genes including *ATM* and *SF3B1*. This suggested an interesting trend towards improved efficacy of olaparib in patients whose tumour was predicted to have a defective DNA damage response due to mutation within *ATM* or *SF3B1*. This was observed as an increased

time on treatment and increased survival time, although larger trials would be required to confirm this observation. In addition, it is possible that PARP inhibition could provide benefit in patients in whom no impairment of the DNA damage is observed within tumour cells as a bulk population. In particular, the importance of clonal heterogeneity and tumour evolution are recognised increasingly in haematological malignancies and it is likely that many patients harbour a minor tumour subclone with mutations within DNA damage response genes. In such a setting the incorporation of olaparib into standard chemotherapy regimens may act to prevent the selection of this potentially resistant subpopulation.

In summary, this early phase trial demonstrates that single agent olaparib is tolerable in patients with haematological malignancies although myelosuppression appears to be an important issue. Future studies would be needed to better define the optimal dosage but this early data suggest that olaparib could have potential clinical utility in patients with DDR defective and clinically refractory haematological malignancies.

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Conflict of Interest:

The authors report no potential conflicts of interest.

Author Contributions:

G.P., C.Y. and T.S., designed the research and G.P was Chief Investigator for the trial. MG, MD, CF, DO, AP, EM, SD, DA. AB, PH, GF, SR were Principal Investigators for the trial. C.Y and D.S conducted the statistical analyses. G.P., C.Y., C.O., D.S. and T.S. interpreted the results. G.P., C.Y., D.S., C.O., R.B., P.M. and T.S were involved in writing the draft manuscript and approved the final manuscript.

Figure legends:

Figure 1. Enrollment, Sequential assignment and Dose Limiting Toxicities experienced by patients for Capsule and Tablet formulations

Figure 2. Overall survival of patients enrolled on the PICCLe trial was unaffected by treatment formulation

Kaplan Meier curves depicting OS of (Figure 2a) all 15 patients and (Figure 2b) stratified according to the treatment formulation using 200 mg or 400 mg capsule (n=9) or 100 mg tablet (n=6) with the number of patients at risk at different time points noted. Median OS was 129, 106 and 129 days; respectively.

Figure 3. Patients enrolled on the PICCLe trial with tumours harbouring CLL driver mutations which play a role in DDR have a slightly elongated time to olaparib discontinutation and longer overall survival

Heatmap depicting the distribution and allelic frequency of genetic alterations in 6 CLL driver genes: *ATM, SF3B1, TP53, BIRC3, NOTCH1* and *MyD88*, aligned according to time on olaparib treatment. Patients who sustained treatment with olaparib for at least 2 months are enriched for the mutated DDR genes, ATM and/or SF3B1. *Indicates the presence of an 11q deletion, NOTCH1 AF estimated from Sanger sequencing (Figure 3a). Kaplan-Meier curves depicting (Figure 3b) time to treatment discontinuationt and (Figure 3c) OS for patients according to the presence (n=9) or absence (n=6) of a DDR defect with the number of patients at risk at different time points noted. There is a non-significant trend towards elongation of both treatment duration (83 *vs* 37.5 days) and OS (192 *vs* 89 days) for patients harbouring mutation(s) of the DDR genes, *ATM* and *SF3B1* (DDR mutated) *vs* those lacking a mutation of these two DDR genes (DDR unmutated).

Table Legends:

Table 1: Baseline Summary Statistics of Patient Characteristics for both the Capsule and Tablet Formulations of Olaparib

This table contains counts and percentages for categorical patient characteristics (e.g. Gender, Disease) and contains the Mean (sd), Median and Range for continuous Patient Characteristics at baseline (e.g. Time from Diagnosis, Age).

Table 2: Trial Patient Summary

This table provides information at the patient level on both patient characteristics (e.g. age, gender, disease) and key trial information (e.g. DLT status, Discointuation reason and mutation status).

Table 3: Adverse events: all grades and grade >= 3

Details the number of patients who have experienced adverse events categorised using the CTCAE system. The number of patients are provided for experiencing a particular event at any grade and grade ≥ 3 for each dose given to patients for both the capsule and tablet formulations.

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