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Chromosome aberrations determined by sFISH and G-banding in lymphocytes from workers with internal deposits of plutonium

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ABSTRACT

Purpose: To examine the influence of α-particle radiation exposure from internally deposited plutonium on chromosome aberration frequencies in peripheral blood lymphocytes of workers from the Sellafield nuclear facility, UK. **Materials and methods**: Chromosome aberration data from historical single colour fluorescence *in situ* hybridization (sFISH) and Giemsa banding (G-banding) analyses, together with more recent sFISH results, were assessed using common aberration analysis criteria and revised radiation dosimetry. The combined sFISH group comprised 29 men with a mean internal red bone marrow dose of 21.0 mGy and a mean external γ-ray dose of 541 mGy. The G-banding group comprised 23 men with a mean internal red bone marrow dose of 23.0 mGy and a mean external γ-ray dose of 315 mGy. **Results**: Observed translocation frequencies corresponded to expectations based on age and external γ-ray dose with no need to postulate a contribution from α-particle irradiation of the red bone marrow by internally deposited plutonium. Frequencies of stable cells with complex aberrations, including insertions, were similar to those in a group of controls and a group of workers with external radiation exposure only, who were studied concurrently. In a similar comparison there is some suggestion of an increase in cells with unstable complex aberrations and this may reflect recent direct exposure to circulating lymphocytes. **Conclusions**: Reference to *in vitro* dose response data for the induction of stable aberrant cells by α-particle irradiation indicates that the low red bone marrow α-particle radiation doses received by the Sellafield workers would not result in a discernible increase in translocations, thus supporting the *in vivo* findings. Therefore, the greater risk from occupational radiation exposure of the bone marrow resulting in viable chromosomally aberrant cells comes from, in general, much larger γ-ray exposure in comparison to α-particle exposure from plutonium.

Introduction

Chromosome analysis in peripheral blood lymphocytes following exposure to low Linear Energy Transfer (LET) radiation has become an established tool for estimating radiation dose, with dicentrics being the aberration of choice for recent exposures, and translocations, which can survive repeated cell division, providing a quantifiable marker of past accidental acute exposure and an integrated measure of chronic exposure (Edwards et al. 2005; 2007; Ainsbury et al. 2011; International Atomic Energy Authority [IAEA] 2011). Interpretation of chromosome aberration frequencies following exposure to high LET α-particles from internally deposited radionuclides has been more difficult. Early studies of plutonium workers using Giemsa banding (G-banding), which produces a unique pattern of bands along each chromosome pair, reported increases in exchange aberrations, most notably

symmetrical exchanges, i.e. translocations, but the workers were grouped according to body burdens of plutonium, and different modes of intake make it difficult to quantify the effect (Brandom et al. 1979; 1990; Tawn et al. 1985; Whitehouse et al. 1998). Nevertheless, the increases in translocations in peripheral blood lymphocytes did not appear to be accounted for by their associated external exposure to γ-radiation. A further study, also based on plutonium body burdens, which applied fluorescence *in situ* hybridization (FISH) techniques to the analysis of chromosome aberrations, reported a greater frequency of translocations in a group of plutonium workers in comparison with a group of workers with only external γ-ray exposure, but in this study the increase was attributed to the greater external exposure received by the plutonium worker group (Salassidis et al. 1998). More recent worker studies using FISH have recognized that it is direct exposure of the haemopoietic stem cells

which will influence chromosome aberration frequencies in peripheral blood lymphocytes and have, therefore, used cumulative red bone marrow α-particle radiation doses from internally deposited plutonium as the measure of exposure (Hande et al. 2003; Livingston et al. 2006; Tawn et al. 2006; Sotnik et al. 2014). Three of these studies (Hande et al. 2003; Livingston et al. 2006; Tawn et al. 2006) reported significant increases in translocation frequencies associated with red bone marrow α-particle radiation doses. The presence of complex stable aberrations was also noted (Livingston et al. 2006; Tawn et al. 2006; Sotnik et al. 2014) although these were at much lower frequencies.

While *in vivo* studies highlight translocations as the predominant aberration increased in radiation workers with plutonium exposure, *in vitro* studies illustrate the complexity of the chromosome rearrangements resulting from α-particle radiation exposure (Griffin et al. 1995; Anderson et al. 2000; 2002; 2006; Moquet et al. 2001; Barquinero et al. 2004; Tawn et al. 2007; Curwen et al. 2012). The spectrum of chromosome aberrations induced by radiation is influenced by the qualitative structure of the radiation track which dictates both the nature of the DNA damage and the proximity of the lesions (Hill et al. 2011; Pignalosa and Durante 2011). Exposure to sparsely ionizing low LET radiation results in a relatively homogeneous spatial distribution of DNA damage across the cell, and at low doses will result in aberrant cells containing a single simple interchange, e.g. a dicentric or a translocation. In contrast, a densely ionizing α-particle will only intersect a small fraction of the cell volume resulting in highly localized deposition of energy and much more clustered DNA damage. This will result in exchanges both between and within traversed chromosomes leading to multiple rearrangements from a single track. This qualitative difference in the types of aberration induced by low as opposed to high LET radiation led to the search for a chromosome marker specific for high LET radiation which could be used to identify and quantify *in vivo* exposure.

Because of the highly localized nature of the DNA damage produced by α-particle radiation, multiple breaks often occur within a single chromosome resulting in predictions that intra-chromosomal rearrangements such as inversions could be potential markers of *in vivo* exposure (Brenner and Sachs 1994). Support for this came from multi-coloured banding (mBAND) studies of workers from the Russian Mayak nuclear facility that reported significant increases in chromosome intra-changes in highly exposed plutonium workers in comparison with a group of workers with just external γ-ray exposure (Hande et al. 2003; Mitchell et al. 2004). However, the application of mBAND to the analysis of aberrations induced *in vitro* by α-particle radiation demonstrated that most intra-changes are associated with complex rearrangements and thus unlikely to be transmissible through cell division (Tawn et al. 2008). The possibility of a stable chromosomal marker specific to high LET radiation exposure was also raised when early *in vitro* studies of chromosome aberrations induced by α-particle radiation highlighted the production of insertions (Anderson et al. 2000; 2003). Subsequent more detailed *in vitro* analyses revealed that, like inversions, most insertions are part of complex unstable

aberrations and when analysis was restricted to stable cells very few were observed (Barquinero et al. 2004; Tawn et al. 2007; Curwen et al. 2012). Nevertheless, the application of multi-coloured FISH (mFISH) has shown that while the majority of α-particle-irradiated cells will suffer complex non-transmissible chromosome damage, a significant proportion survive with simple exchanges, in particular translocations, that can be passed on to descendant cells (Anderson et al. 2005; Curwen et al. 2012) thus giving credence to the findings in the worker studies.

The application of FISH translocation analysis for assessing the extent of long-term exposure to low dose, low dose-rate, low LET radiation can provide a valuable contribution to dose reconstruction for epidemiological studies (Edwards et al. 2007). However, many potentially radioactive environments, particularly those associated with the nuclear industry, can also include exposure from internally deposited radionuclides and translocation frequencies in individuals with both internal and external exposure can be difficult to interpret (Vozilova et al. 2012; Ainsbury et al. 2014). This laboratory has recently derived an *in vitro* dose response for translocations in stable cells following exposure to α-particle radiation (Curwen et al. 2012). This has been used, together with an established dose response for γ-ray exposure, to derive expected values for translocation frequencies based on recorded doses for a group of workers from the Russian Mayak nuclear facility with lifetime exposures to γ-radiation and mixed γ-radiation and α-particle radiation (Curwen et al. 2015). The results of this pilot study were encouraging, leading to the conclusion that translocation frequency can be used to validate red bone marrow doses resulting from mixed exposure to external and internal radiation. Here we report the recent analysis of chromosome aberrations in a group of plutonium workers from the Sellafield nuclear facility and bring together the results of sampling over a 30-year period.

Materials and methods

Study group

The male plutonium workers included in this report were sampled in five time periods over a 30-year timespan. For the purposes of this analysis all doses at time of sampling have been reassessed. Total cumulative occupational doses to the red bone marrow from external sources of radiation were derived from film badge readings (Kite and Britcher 1996) and cumulative internal absorbed doses from plutonium exposures were assessed from urinalysis results using mathematical models of intake, metabolism and excretion (Riddell et al. 2015). The internal dose assessment methodology used included the revision of the International Commission on Radiological Protection (ICRP) Publication 67 (ICRP 1993) systemic model for plutonium described by Leggett et al. (2005), likely to form the basis of the next ICRP model, that is considered to be the most accurate predictor of actual plutonium metabolism yet developed.

Earlier analyses involving the Sellafield worker cohort relied on equivalent doses from plutonium, recorded in milli-sieverts (mSv). Equivalent dose incorporates a radiation weighting

factor of 20 for doses from α-particles (ICRP 2007). This reflects the potentially greater risks from exposure to this type of radiation and includes an element of caution as this quantity was developed for radiation protection purposes. It has since been recognized that using equivalent doses for research can prevent an unbiased assesment of the relative biological effectiveness of α-particle radiation, as compared to γ-radiation, for any specific endpoint under study. Consequently, the doses calculated and used in this and other more recent analyses had been absorbed doses (i.e. they did not include any radiation weighting factor), reported in milligray (mGy). Only those with internal red bone marrow α-particle doses > 10 mGy were included in this analysis. This was a higher cut-off point than used in previous reports and was chosen because recent *in vitro* (Curwen et al. 2012) and *in vivo* (Curwen et al. 2015) studies in this laboratory indicated that doses <10 mGy are likely to result in <0.5 translocation per 1000 cells.

Group 1 comprised 11 workers sampled in 1978–1982, and analyzed with G-banding, who were first reported as part of a larger group of 54 plutonium workers identified at the time as having Pu body burdens >296 Bq (Tawn et al. 1985). Group 2 comprised eight of these workers who were resampled in 1986–1991. This second study also used G-banding and the chromosome aberration frequencies were again assessed in relation to Pu body burdens (Whitehouse et al. 1998), and later in relation to red bone marrow α-particle doses (Tawn and Whitehouse 2005). Group 3 comprised four plutonium workers who provided samples for G-banding analysis in 1993–1994, three of whom had also provided samples for Groups 1 and 2. The samples in Group 3 were taken as part of a larger study of radiation workers, the vast majority of whom were exposed to external radiation and had received negligible Pu exposure. Results on chromosome analysis using G-banding (Whitehouse et al. 1998) and FISH (Tucker et al. 1997) in those with external exposure are available, but the G-banding results on the plutonium workers sampled at this time have not previously been reported. The 22 men who comprised Group 4 were sampled in 1996–2003 and formed part of the first group of Sellafield plutonium workers analyzed with single colour FISH (sFISH) (Tawn et al. 2006). Group 5 comprised seven new samples taken in 2004–2008 as part of a wider investigation of Sellafield workers and their families. Full details of enrolment and sample collection for this most recent collection have been reported (Tawn et al. 2015a; 2015b). Briefly, a presentation was made to workforce representatives at the Sellafield facility and, having gained their support, ethical approval was obtained from North Cumbria Local Research Ethics Committee. Letters, including information sheets and consent forms, were sent to potential participants and those agreeing to participate were visited by a genetic nurse who took blood samples.

Lymphocyte culture

Whole blood lymphocyte cultures were set up for 47–48 h and followed standard procedures recommended by the International Atomic Energy Authority (IAEA 2011). In order to check cell cycling times, bromodeoxyuridine (BrdU) (Sigma, Poole, Dorset, UK) was added to the culture medium and fluorescence plus Giemsa staining undertaken. This was routinely done for all cultures for Groups 1, 2 and 3 and periodically for Groups 4 and 5.

Single colour fluorescence *in situ* hybridization (sFISH)

sFISH was performed on metaphase spreads using a cocktail of biotinylated probes for either chromosomes 1, 3 and 4 or chromosomes 1, 3 and 5 and a pancentromeric probe directly labelled with Streptavidin-Cyanine 3 (Cy3) (Metasystems, Altlussheim, Germany) as previously described (Bothwell et al. 2000; Thierens et al. 2005). Briefly, slides were aged for 2–3 d at room temperature before being denatured in 70% formamide/0.03 M sodium citrate, 0.3 M sodium chloride, pH 7 (2 × SSC) (Sigma-Aldrich Company Ltd, Dorset, UK) at 65 °C. Following denaturation of the chromosome (65 °C) and pancentromeric probes (85 °C), both were applied together to the denatured slides and allowed to hybridize overnight. Excess paint was subsequently washed off and the biotinylated chromosome probes detected with alternate layers of 4 μg ml^{-1} avidin-fluorescein isothiocyanate (avidin-FITC) (Metasystems) and 5 μg ml^{-1} biotinylated goat anti-avidin (Metasystems). The chromosomes were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI) (Metasystems) in Vectashield anti-fade solution (Metasystems). Analysis was carried out using a Nikon microscope with epifluorescence attachment and a computerized image analysis system (ISIS Metasystems) was used to capture and confirm all aberrations.

Only intact cells with ∼46 chromosomes and all painted material present were included in the analysis. All aberrations were recorded and described according to the Protocol for Aberration Identification and Nomenclature Terminology (PAINT) scheme (Tucker et al. 1995). Simple exchanges, e.g. translocations and dicentrics, were recorded as two-way or one-way but, for the purposes of this analysis, one-way and two-way exchanges were added together. Rearrangements involving three or more breaks in two or more chromosomes were recorded as complex. Stable complexes were examined for the number of insertions and unstable complexes for the number of dicentric equivalents. Counterstained chromosomes in all cells with a painted rearrangement were examined for the presence of other aberrations in order to help identify complex aberrations and to establish stability status. Cells with dicentrics, centric rings and acentric fragments were classed as unstable whereas cells with only monocentric chromosomes were classed as stable. Where rogue cells were observed these were recorded separately and no attempt was made to resolve the aberrations. Such cells were placed in the unstable complex cell category. The presence of two or more cells with identical aberrations was considered as evidence of a clone and the aberrant cell only scored once.

G-banding

Slides were G-banded with trypsin to a resolution of approximately 400 bands per cell (Seabright 1971). Chromosome

analysis was performed using a standard light microscope and 100 cells per individual were karyotyped. For this report, the classification of aberrations has been reviewed.

Results

Data on the individuals in each sampling time and the staining techniques employed are provided in Table 1. Table 2 presents the sFISH aberration data for Groups 4 and 5 and the two groups combined. FISH analysis of bicoloured aberrations detects only a proportion of the total aberrations present in a cell, and translocation and dicentric frequencies are converted to whole genome equivalents using the Lucas formula (Lucas et al. 1992). Extrapolation to the whole genome is not appropriate for complex aberrations and therefore complex stable and unstable frequencies are provided for total cells scored. The re-assessed G-banding data on Groups 1, 2 and 3 are presented in Table 3. However, when reviewing the G-banding data and the analysis procedures in place at the time, it became apparent that cells with complex aberrations that could not be resolved would sometimes be excluded from the analysis on the assumption that these were rogue cells. This is likely to have led to an under-ascertainment of complex cells, both stable and unstable. Therefore in this reanalysis of G-banding results only data on cells with translocations and dicentrics are presented. Table 4 presents a comparison of complex numbers and frequencies with those recently reported in the sFISH study of Sellafield workers with external γ-ray exposure (Tawn et al. 2015b).

Discussion

The frequency of translocations in peripheral blood lymphocytes increases with age and the effect has been quantified in two large international studies utilizing FISH techniques (Whitehouse et al. 2005; Sigurdson et al. 2008). In the current analysis of the plutonium worker sFISH results (Table 2) we chose to derive expectations for age based on the larger study by Sigurdson et al. (2008), that incorporates data from the earlier study by Whitehouse et al. (2005), rather than rely on a small number of matched controls as was done in previous reports. Sigurdson et al. (2008) provides methodology for the calculation of absolute expected translocation rates taking into account age, gender, race, smoking status and laboratory origin and this has been adopted in recent studies of radiation-exposed populations to produce age-adjusted rates (Bhatti et al. 2010; Sotnik et al. 2014). Using the same approach, the expected age-related mean translocation frequency for the group of 29 plutonium workers analyzed with sFISH (Table 2) was 13.78×10^{-3} per cell.

The dose response for the induction of translocations by low LET γ-irradiation has been characterized by *in vitro* studies and translocation frequency is an established measure for estimating dose following acute accidental over-exposures (IAEA 2011). Theoretical considerations indicate that translocation frequencies resulting from chronic exposure to low dose low LET radiation should conform to the linear component of the linear-quadratic model applied to *in vitro* acute high dose

studies. For high energy γ-irradiation this is ∼1.5 per 100 genome equivalent cells per Gy (Edwards et al. 2005). Studies of workers exposed occupationally at the Sellafield nuclear facility to external γ-radiation have confirmed a linear dose response with the most recent and most comprehensive resulting in a dose response of $1.16 \pm 0.16 \times 10^{-2}$ translocations per genome equivalent cells per Gy (Tawn et al. 2015b). The plutonium workers in the present report had been exposed to similar external radiation conditions as their colleagues and we therefore chose to use the dose response derived from the Sellafield workers for external γ-irradiation to estimate the expected translocation frequency from their external exposure. The mean external dose for the combined study Groups 4 and 5 of 29 plutonium workers examined with sFISH was 541 mGy, which is expected to give a yield of translocations of $6.28 \pm 0.87 \times 10^{-3}$ per genome equivalent. This, added to the age expectation of 13.78×10^{-3} per cell, gave an expected translocation frequency of $20.06 \pm 0.87 \times 10^{-3}$ per genome equivalent. The observed translocation frequency of $18.93 \pm 1.24 \times 10^{-3}$ per genome equivalent cells was, therefore, in line with expectations based purely on age and external γ-ray dose and left little room for a contribution from internal α-particle exposure of the red bone marrow. This is not unexpected, as reference to the *in vitro*-derived dose response for translocations in stable cells induced by α-particle radiation of $4.1 \pm 1.3 \times 10^{-2}$ per Gy (Curwen et al. 2012) indicates that a dose of 20.4 mGy would result in a translocation frequency of $0.84 \pm 0.27 \times 10^{-3}$ per genome equivalents.

In a similar analysis of historical G-banding data (Table 3) we derived an age-expected translocation frequency for the combined group of 23 plutonium workers of 9.41×10^{-3} translocations per cell and an expected frequency of $3.65 \pm 0.50 \times 10^{-3}$ translocations per cell for the mean external γ-ray dose of 315 mGy. Together this gave an expectation of $13.06 \pm 0.50 \times 10^{-3}$ translocations per cell which was within the range of the observed frequency of $11.3 \pm 2.22 \times 10^{-3}$ translocations per cell. Therefore, as with the sFISH analysis, it is not necessary to postulate any contribution from α-particle irradiation of the red bone marrow.

In previous reports of chromosome aberrations in plutonium workers from Sellafield we have suggested that the exposure from intakes of plutonium could result in a measurable increase in chromosome aberrations in peripheral blood lymphocytes (Tawn et al. 1985; 2006; Whitehouse et al. 1998; Tawn and Whitehouse 2005). Since then more information on the nature of aberrations induced by both γ-radiation and α-particle radiation has led to a better understanding of the profile of aberrant cells likely to survive cell division and thus acummulate with ongoing chronic occupational exposure. For γ-radiation, this is a cell with a single translocation (Tawn et al. 2015b). For α-particle radiation a much greater amount and complexity of chromosomal damage is initially induced, but the vast majority of this damage results in unstable cells which will not successfully undergo cell division and it is stable cells with a simple translocation that are most likely to survive (Anderson et al. 2005; Tawn et al. 2007; Curwen et al. 2012). Even so, the *in vitro*-derived dose response for translocations in stable cells of $4.1 \pm 1.3 \times 10^{-2}$ per Gy for α-particle

Table 1. Individual data on date of birth, smoking status, analysis technique, sampling times and red bone marrow doses from internal plutonium α-particle irradiation and external γ-irradiation.

			Red bone marrow dose from: internal plutonium α-particles (mGy), and external γ-rays (mGy)				
Sample ID	Date of birth	Smoking status	Group 1 G-band 1978–1982	Group 2 G-band 1986–1991	Group 3 G-band 1993–1994	Group 4 sFISH 1996–2003	Group 5 sFISH 2004–2008
1	04/09/1924	S	19.2 145.6				
2	12/01/1923	S	10.7 825.4				
3	21/04/1921	S	33.2 289.7				
4	11/11/1919	S	12.8 240.6				
5	11/04/1921	N	41.0 453.8				
6	28/06/1927	S	17.2 418.6				
7	25/11/1924	N/S	13.2 232.1	16.3 303.7			
8	26/01/1942	S	14.8 332.7	23.3 342.2	27.0 344.1		
9	16/08/1929	N/N/S	15.6 472.7	24.0 472.7	27.7 472.7		
10	18/05/1929	S	43.1 112.6	50.8 112.6	54.3 112.6	57.7 112.6	
11	05/03/1935	N	15.3 317.2	24.8 322.8		32.1 325.1	36.0 325.1
12	19/11/1928	S		11.9 547.9			
13	18/12/1928	S		11.70 133.2			
14	23/12/1936	S		10.4 37.0		13.6 37.0	
15	16/12/1932	S			11.0 211.0		
16	17/03/1921	S				14.8 225.0	
17	08/04/1941	S				11.4 594.1	
18	25/07/1918	S				16.2 236.0	
19	09/06/1928	S				12.7 649.6	
20	16/11/1919	S				25.2 197.6	
21	19/06/1925	S				22.1 304.5	
22	30/03/1927	N				15.6 684.2	
23	21/04/1923	S				15.8 740.0	
24	26/06/1929	N				11.5 1336.3	
25	06/10/1929	S				38.1 209.3	
26	29/06/1926	S				10.1 726.4	
27	03/06/1929	S				10.1 486.5	
28	19/04/1936	S				13.3 745.1	
29	07/08/1928	S				11.6 775.8	
30	09/12/1924	S				14.2 624.0	
31	01/02/1924	S				24.1 296.5	
32	11/11/1926	S				15.6 285.3	
33	04/06/1925	S				22.8 811.1	24.7 811.1
34	01/11/1930	S				40.7 1077.1	46.5 1077.1
35	16/04/1927	S					12.7

(continued)

Table 1. Continued

			Red bone marrow dose from: internal plutonium α-particles (mGy), and external γ-rays (mGy)				
Sample ID	Date of birth	Smoking status	Group 1 G-band 1978–1982	Group 2 G-band 1986–1991	Group 3 G-band 1993–1994	Group 4 sFISH 1996–2003	Group 5 sFISH 2004–2008
36	16/08/1929	S					704.1 35.6
37	15/04/1931	S					472.7 17.3
38	17/08/1950	S					408.5 10.3 401.2

N: non-smoker; S: ever smoker.

Table 2. Data on age, smoking, red bone marrow dose and chromosome aberration frequencies assessed using sFISH.

	Group 4	Group 5	Combined
Number of individuals	22	7	29
Mean age (years) (range)	71 (57–81)	72 (56–79)	71 (56–81)
Number of ever smokers (%)	19 (86.4)	6 (85.7)	25 (86.2)
Mean internal α-particle dose (mGy) (range)	20.4 (10.0–57.7)	26.1 (10.3–46.5)	21.8 (10.0–57.7)
Mean external γ-ray dose (mGy) (range)	522 (37–1336)	600 (325–1077)	541 (37–1336)
Number of cells scored	26400	9528	35928
Number of genome equivalents	9105	3259	12364
Number of translocations	170	64	234
Translocations per genome equivalent $\pm$ SE $\times$ 10^{-3}	18.67 $\pm$ 1.43	19.64 $\pm$ 2.46	18.93 $\pm$ 1.24
Number of dicentrics	10	4	14
Dicentrics per genome equivalent $\pm$ SE $\times$ 10^{-3}	1.10 $\pm$ 0.35	1.23 $\pm$ 0.61	1.13 $\pm$ 0.30
Number of dicentric equivalents	2	7	9
Dicentrics + dicentric equivalents per genome equivalent $\pm$ SE $\times$ 10^{-3}	1.32 $\pm$ 0.38	3.38 $\pm$ 1.02	1.86 $\pm$ 0.39
Number of unstable complexes (rogue cells)	18 (1)	8 (1)	26 (2)
Unstable complex aberrations per total cells $\pm$ SE $\times$ 10^{-3}	0.68 $\pm$ 0.16	0.84 $\pm$ 0.30	0.72 $\pm$ 0.14
Number of stable complexes (insertions)	7 (6)*	6 (1)	13 (7)[a]
Stable complex aberrations per total cells $\pm$ SE $\times$ 10^{-3}	0.27 $\pm$ 0.10	0.63 $\pm$ 0.26	0.36 $\pm$ 0.10

[a]Two insertions present in one cell as part of one complex aberration.

Table 3. Data on age, smoking, red bone marrow dose and simple chromosome aberration frequencies assessed using G-banding.

	Group 1	Group 2	Group 3	Combined
Number of individuals	11	8	4	23
Mean age (years) (range)	53 (38–61)	57 (47–63)	61 (52–64)	56 (38–64)
Number of ever smokers (%)	7 (63.6)	6 (75.0)	4 (100.0)	17 (73.9)
Mean internal α-particle dose (mGy) (range)	21.5 (10.7–43.1)	21.7 (10.4–50.8)	30.0 (11.0–54.3)	23.0 (10.4–54.3)
Mean external γ-ray dose (mGy) (range)	349 (113–825)	284 (37–548)	285 (113–473)	315 (37–825)
Number of cells scored	1100	800	400	2300
Number of translocations	10	13	3	26
Translocations per cell $\pm$ SE $\times$ 10^{-3}	9.09 $\pm$ 2.87	16.25 $\pm$ 4.51	7.50 $\pm$ 4.33	11.30 $\pm$ 2.22
Number of dicentrics	2	2	0	4
Dicentrics per cell $\pm$ SE $\times$ 10^{-3}	1.82 $\pm$ 1.29	2.50 $\pm$ 1.77	0	1.74 $\pm$ 0.87

Table 4. Comparison of complex aberrations determined by sFISH.

	Controls[a]	External workers 50–499 mGv[a]	External workers > 500 mGy[a]	Pu workers
Number of individuals	97	247	115	29
Mean internal α-particle dose (mGy) (range)	0.4 (0–4.5)	1.8 (0–9.4)	3.3 (0–9.6)	21.8 (10.0–57.7)
Mean external γ-ray dose (mGy) (range)	22 (0–50)	267 (57–499)	687 (500–1563)	541 (37–1336)
Number of cells scored	132700	271709	131418	35928
Number of unstable complexes (rogue cells)	59 (16)	96 (12)	78 (25)	26 (2)
Unstable complex aberrations per total cells $\pm$ SE $\times$ 10^{-3}	0.44 $\pm$ 0.06	0.35 $\pm$ 0.04	0.59 $\pm$ 0.07	0.72 $\pm$ 0.14
Number of stable complexes (insertions)	47 (27)	59 (32)[b]	60 (45)	13 (7)[b]
Stable complex aberrations per total cells $\pm$ SE $\times$ 10^{-3}	0.35 $\pm$ 0.05	0.22 $\pm$ 0.03	0.46 $\pm$ 0.06	0.36 $\pm$ 0.10

[a]Data from Tawn et al. (2015b). [b]Two insertions present in one cell as part of one complex aberration.

radiation (Curwen et al. 2012) was still four times greater than that derived for occupational chronic exposure to γ-radiation of $1.16 \pm 0.16 \times 10^{-2}$ per Gy (Tawn et al. 2015b). However, since the doses received by the men in this study from α-particle radiation were low, i.e. in the tens of mGy, it is not surprising that little, if any, of the translocations observed in

peripheral blood lymphocytes can be attributed to α-particle radiation exposure. A reappraisal of the earlier studies of Sellafield plutonium workers which used red bone marrow doses (Tawn and Whitehouse 2005; Tawn et al. 2006) suggested that although translocation frequencies in plutonium workers were higher than in comparable workers with similar recorded external exposure but negligible internal exposure, it was the lower frequencies in the workers with solely external exposure which were unusual, being much lower than expected and little different to those reported for controls.

In a study of workers from the Russian Mayak facility, Hande et al. (2003) compared workers with mixed exposure to γ-radiation and α-particle radiation with workers with just exposure to γ-radiation and demonstrated a marked increase in translocation yield associated with the red bone marrow α-particle dose. However, in this study the doses were high, being 2.3 Gy of γ-radiation for the workers with only external exposure, and a combination of 1.5 Gy external γ-radiation and 1.1 Gy internal α-particle radiation exposure for the men with mixed exposure. The α-particle radiation dose was 1.5–2.0 orders of magnitude greater than those reported for the Sellafield workers and based on the *in vitro* dose response for translocations in stable cells (Curwen et al. 2012) such a dose would be expected to yield 45 translocations per 10^{-3} cells, thus supporting these findings. In contrast, Sotnik et al. (2014), who also studied workers from the Mayak facility with mixed γ-radiation and α-particle radiation exposure, found a significant correlation between translocation yield and external γ-ray exposure, but no correlation with Pu body burden or internal red bone marrow α-particle radiation dose. In this group of plutonium workers the internal doses ranged from 0–0.84 Gy (mean 0.1 Gy). Based on *in vitro* expectations an α-particle dose of 0.1 Gy would yield ~4 translocations per 10^{-3} cells and could be discernible. Moreover, division into two dose groups based on body burdens revealed a higher translocation frequency in the group with >1.48 kBq Pu body burden in comparison with the group with <1.48 kBq. However, since no information on red bone marrow internal α-particle doses or external γ-ray doses for these two groups was provided, it is not possible to estimate the expected contribution made by the two types of radiation exposure to the translocation yield. A US study of workers from the Rocky Flats Plant, Colorado, USA (Livingston et al. 2006) is also difficult to interpret because the control translocation frequency was lower than expected, being half that derived from international studies of background frequencies (Whitehouse et al. 2005; Sigurdson et al. 2008). The low dose group with external γ-ray exposure had a frequency commensurate with age and, since the median dose for this group of 22 mSv did not give a measurable increase in translocation frequency, this is in line with expectation. The plutonium worker group was older and had a median external dose of 280 mSv. Deriving expectations based on age and external dose leaves some excess of translocations which supports the authors' conclusion that α-particle exposure is contributing to translocation yield. However, the reported median internal red bone marrow dose of 168 mSv equates to only 8 mGy absorbed

dose (assuming a radiation weighting factor of 20) making it difficult to envisage a quantifiable effect.

It is clear that a better understanding of the dose response for the induction of translocations in stable cells by α-particle radiation leads to a reconciliation of the conflicting results of previous studies of chromosome aberrations in peripheral blood lymphocytes of plutonium workers. These can be explained, at least in part, by the large differences in red bone marrow α-particle radiation doses received by the plutonium workers in the different studies. Thus, in the study of Mayak plutonium workers with a mean red bone marrow α-particle dose of 1.1 Gy, a clear increase in translocation frequencies commensurate with dose was demonstrated (Hande et al. 2003), whereas for the Sellafield plutonium workers reported here, with a mean red bone marrow α-particle dose of 20.4 mGy, no increase in translocation frequencies associated with plutonium exposure was observed. In line with this, a recent pilot study of Mayak workers with mixed exposures, analyzed with mFISH, examined individual translocation yields in relation to expectations based on *in vitro* dose response relationships for translocations in stable cells and confirmed that α-particle radiation doses > 100 mGy received by the red bone marrow can be discernible (Curwen et al. 2015).

Previous *in vitro* work in this laboratory using sFISH (Tawn et al. 2007) and mFISH (Curwen et al. 2012) demonstrated that α-particle radiation can induce stable aberrant cells containing multiple/complex rearrangements, but such cells are at a much lower frequency than cells containing a single translocation. The frequency of stable cells with complex aberrations, and the proportion of stable complexes involving insertions, in the present study of plutonium workers, was within the range observed in the workers with exposure to external γ-radiation and was the same as the control frequency (Table 4). This is not surprising in light of the lack of an increase in translocations. Unstable cells with complex aberrations were slightly raised over both controls and those with only external γ-ray exposure (Table 4), but in a comparison with the group of external workers with γ-ray doses >500 mGy this is not significant ($\chi_1^2 = 0.57$, $p = 0.45$). However, this may reflect some recent direct exposure of peripheral blood lymphocytes to α-particle radiation which was not included in estimates of red bone marrow dose. In line with this is the finding that dicentrics occurred at a frequency of $1.13 \pm 0.30 \times 10^{-3}$ per cell (Table 2) which corresponds to accepted background frequencies of one dicentric in 1000–2000 cells (Edwards et al. 2007; IAEA 2011). When dicentric equivalents were included, the frequency increased to $1.86 \pm 0.39 \times 10^{-3}$ per cell (Table 2) which reflects the presence of unstable complex cells. The dicentric frequency derived with G-banding was $1.74 \pm 0.87 \times 10^{-3}$ per cell (Table 3) which was not significantly different from the dicentric frequency of $1.54 \pm 0.34 \times 10^{-3}$ per cell which can be derived from the concurrent G-banding study of background aberration frequencies (Tawn and Whitehouse 2001).

It is now apparent that for the Sellafield plutonium workers, the greater risk of occupational radiation exposure of the bone marrow resulting in viable chromosomally aberrant cells comes from the much larger external γ-ray exposure rather than internal α-particle exposure from plutonium.

The α-particle radiation exposure of the bone marrow received by these men will have resulted in the majority of the cells hit undergoing cell death. This effect would only become important if sufficient were killed to result in clinically relevant depletion of blood cells and/or some sort of proliferative stress as the remaining stem cells seek to replenish the lost cells. However, α-particle radiation doses to Sellafield plutonium workers are evidently far too low for this to be a consideration. Molecular epidemiological studies have shown an association between an increased load of chromosomal aberrations in peripheral blood lymphocytes in healthy individuals and an increased risk of cancer (Bonassi et al. 2008). Indeed, to date, chromosomal aberrations are still recognized as the only validated biomarker of cancer risk (Durante et al. 2013). It is therefore of note that while epidemiological studies of Sellafield workers have demonstrated an increase in leukaemia incidence in relation to external γ-irradiation, no such increase has been found for exposure to internally deposited plutonium (Omar et al. 1999).

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