m-FISH analysis reveals complexity of chromosome aberrations in individuals occupationally exposed with internal plutonium: A pilot study to assess the relevance of complex aberrations as biomarkers of exposure to high-LET α-particles.

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We recently demonstrated that a significant proportion of apparently stable insertions induced after exposure to a mean of 1 α-particle/cell, detected using 3-colour FISH, were part of larger unstable complexes when visualised by 24-colour FISH. Interestingly, regardless of the long-term persistence capability of the cell, the complexity of each α-particle-induced complex appeared to be specific to the nuclear traversal of a single α-particle. To assess whether aberrations of a similar complexity are observed *in vivo* and also to examine the usefulness of detecting such aberrations as a biomarker of chronic exposure to α-particles, we have carried out a limited pilot study of Russian workers with large body burdens of α-particle emitting plutonium (Pu). We found unstable cells containing non-transmissible complex aberrations in all of the Pu exposed subjects analysed by m-FISH. In addition, all of the complexes seen were consistent with those previously observed *in vitro*. Non-transmissible complex aberrations were more common than transmissible-type complexes, consistent with on-going/chronic exposure and insertions were dominant features of both type of complex. Accordingly, this preliminary study supports the proposal that aberration complexity and non-transmissibility are the major cytogenetic features of α-particle exposure that could potentially be exploited as a specific indicator of chronic exposures to high-LET α-particles.

INTRODUCTION

It is now well established that complex chromosome aberrations are induced after exposure to high-LET radiation and that insertions are characteristic features of these aberrations . Insertions are commonly regarded as stable complex events since they can be defined as the products of an interstitial deletion and it's subsequent 'insertion' into a different chromosome or chromosome region. As a consequence, insertions have been proposed as stable indicators of past-exposure to high-LET radiation . As the power of FISH based techniques has increased, enabling increasing numbers of chromosomes to be uniquely painted, it has become clear that the complexity of radiation-induced chromosome aberrations is much greater than previously thought . Importantly, we now know that rearranged chromosomes may be comprised of multiple chromosomes such that portions of different chromosomes can flank individual segments. Although not mechanistically an insertion, the event is clearly visible as such by FISH and can subsequently be scored .

Recently we demonstrated this using the technique of m-FISH . We found that complex chromosome aberrations were predominantly induced in human peripheral blood lymphocytes (PBL) exposed *in vitro* to a low dose of high-LET α-particles and that the majority of insertions visible were part of larger complex aberrations. In the main, these complex aberrations were of the non-transmissible type and consequently, the frequency of stable insertions which persisted through long-term culture was observed to be low (0.5-1.0%) . What these studies also highlighted was that the mechanism of formation of these complex aberrations appeared to be directly related to the structure of the α-particle

track and the geometry of the cell traversed. In other words, there was a suggestion that the complexity of the aberration induced may be quite specific to α-particle exposure.

The long-term persistence of insertions and their usefulness as biomarkers of past exposure to high-LET α-particle radiation is therefore dependent upon the complexity of the 'complete' aberration in the cell, regardless of method used for analysis. Thus, if complex aberrations of a similar complexity to that seen *in vitro* are also observed after exposure to high-LET α-particles *in vivo*, then it would seem unlikely that stable insertions could be efficiently detected long after the exposure . However radon, which is a natural α-emitter present in the environment, is a continual source of high-LET α-particle exposure . Upon inhalation, radon gas is absorbed into the bloodstream and transported to various organs, resulting in a low but significant exposure to the PBL pool. Consequently, α-particle-induced complexes may be produced in PBL throughout the exposure lifetime, which if of a similar specificity as observed *in vitro*, could be in themselves relevant indicators of chronic exposure, regardless of their long-term transmissibility .

To assess the relevance of detecting such aberrations *in vivo*, we have carried out a limited pilot study of Russian Nuclear-Chemical workers who are known to have large internal deposits of α-particle emitting plutonium (Pu). The estimated doses of the subjects studied is clearly in vast excess to that which would be received from environmental exposure to radon and in addition, the metabolism and organ deposition patterns differ for radon and Pu . The principal aim of this study was therefore to assess the efficiency of detecting insertions by both 3-colour FISH and m-FISH methods and to

address the role non-transmissible complexes could play as biomarkers of chronic exposure to high-LET α-particles *in vivo*.

MATERIALS AND METHODS

Study Group

The pilot study comprised four retired male workers of the Russian Nuclear Industry aged between 55 and 63 (subjects 3-6) at the time of study and two regional controls aged 38 (subject 1) and 40 (subject 2) years. Subjects 3-5 were occupationally exposed throughout periods of 7 to 33 years to combined external γ-radiation (range 14.2 – 31.7 cSv) and inhaled plutonium aerosols (^{239}Pu/^{241}Pu), resulting in a chronic and continued internal exposure to α-particle radiation. Subject 6 was recorded as being exposed to external γ-radiation only (~15 cSv). Blood samples were obtained by medical staff at the State Research Centre Institute of Biophysics, Moscow (SRC-IB), during annual health examinations, according to the guidelines of the Russian Health Ministry and the Russian Atomic Ministry. For this study, fixed chromosome preparations from anonymised samples were provided.

Determination of Pu body burdens and dose estimates were carried at SRC-IB on the basis of results of biophysics studies of Pu content in urine provided by the plant's biophysics laboratory from outpatient and inpatient examinations . Dose estimation took into account the duration of the individual working with Pu and the time of the sampling measurements, using an algorithm based on the modern respiratory tract model as

suggested by ICRP . As an example of the time course, one individual started working with Pu exposure in 1963. Urine measurements from 1976 to 1987 suggested that by this time the Pu body burden had reached an approximately constant level and the measurements provided estimates of the variability of urine assays. Since 1995, additional information for dosimetric determination was obtained by the introduction of measurements of ^{241}Am in lungs and bones using a whole-body counter. This permitted more accurate estimation of the mean body burden and of ratios of activity in lungs to bones, which also identifies the route of entry into the body (inhalation or wounds) and provides estimation of the solubility class of the aerosol .

The Pu body burden of subjects ranged from 2.2 to 11.2 kBq. The main route of radionuclides entering the body was chronic inhalation of Pu aerosols throughout the known period of work of the individual under conditions of probable contact with radionuclides. The aerosols had a known ratio of ^{241}Pu/^{239}Pu and a known amount of ^{241}Am forming by decay of ^{241}Pu, uniquely defined by the effective delay time of the products. The chronic inhalation rate of Pu aerosols for each worker decreased approximately exponentially with a half-time of 4 years from 1960-1970 and with a half-time of 8 years thereafter. The Pu aerosols had lognormal size distributions, with particle sizes of 5-10 μm and standard geometric deviation 2.5. They consisted of S-class compounds (slow dissolution) such as metal or Pu oxide. The daughter product ^{241}Am, forming from ^{241}Pu, dissolves in the lung with the Pu-particle solubility rate and therefore is also S-class. Pure compounds of ^{241}Am in aerosols were classified as M-class (medium solubility). After dissolution and dilution into the blood compartment, the Pu and Am were assigned different biokinetic parameters in accordance with ICRP 67 . Based on the

above information, a computer programme formulated at SRC-IB was applied to calculate the Pu body burden and the doses to lung and other organs . Doses to lung and bone marrow for subjects 3-5, respectively, were evaluated to be as follows: 35 and 2 Sv (subject 3); 180 and 10 Sv (subject 4); 10 and 0.2 Sv (subject 5). Clinically, subjects 3-5 were diagnosed with chronic radiation sickness and, additionally, subjects 3 and 4 with pneumosclerosis.

Lymphocyte Culture and Chromosome Preparation

Whole blood was collected and cultured in phytohaemagglutinin supplemented minimum essential medium containing 20% foetal calf serum for 48 h as previously described . 0.2μg/ml demecolcine was added for the final 2.5 h of culture and metaphase cells were collected using standard cytogenetic techniques .

Three-Colour Fluorescence In Situ Hybridisation (FISH)

Fresh slides of metaphase cells were hardened (3:1 methanol:acetic acid for 1 h, dehydrated through an ethanol series (2 mins each in 70%, 70%, 90%, 90% and 100%), baked at 65^{o}C for 20 min, then 10 min in acetone) and pretreated with RNase A (100 μ g/ml in 2xSSC) at 37^{o}C for 1 h. After washing in 2xSSC and PBS, the cells were treated with pepsin (1:20x10^{3} in 10mM HCL) at 37^{o}C for 5-10 min then washed twice in PBS, 50mM $MgCl_2$/PBS, 50mM $MgCl_2$/1% formaldehyde/PBS then PBS before finally dehydrating through an ethanol series (2 mins each in 70%, 70%, 90%, 90% and 100%).

Commercially produced whole chromosome probes for chromosome 1 (FITC:biotin ratio mix), chromosome 2 (FITC) and chromosome 5 (Cy3) were used together with a biotinylated pancentromeric probe (Cambio, UK). *In situ* hybridization was carried out as described previously .

Metaphase chromosomes were analysed on an Olympus fluorescence microscope with filter sets for DAPI, FITC, FITC/DAPI/Texas Red and FITC/DAPI/TRITC. A metaphase was classified as undamaged if neither of the painted homologues of chromosomes 1, 2 or 5 were visibly involved in a chromosome-type exchange.

Multiplex FISH (m-FISH)

Fresh slides of metaphase chromosomes were hardened and pretreated with RNase A and pepsin as described above. For hybridisation, cells were denatured in 70% formamide/2xSSC at 72ºC for 3 min and dehydrated for 1 min each in 70/90/100% ethanol. Parallel to this, the commercially available 24-colour paint cocktail, SpectraVision™ Assay (Vysis, UK), was denatured at 73ºC for 6 min. Cells and probe were then mixed and left to hybridise for 36-48 h at 37ºC before being washed in 0.4xSSC/0.3% Igepal (Sigma, UK) at 71ºC for 2-3 min and in 2xSSC/0.1% Igepal at room temperature for 10 sec. Cells were counterstained using DAPI III (Vysis, UK), sealed and stored in the dark at -20ºC.

Chromosome aberrations were analysed as previously described . In brief, metaphase chromosomes were visualised using a 6-position Olympus BX51 fluorescent microscope containing individual filter sets for each component fluor of the SpectraVision (Vysis

(UK) Ltd) probe cocktail plus DAPI. Digital images were captured for m-FISH using a charged-coupled device (CCD) camera (Photometrics Sensys CCD) coupled to and driven by Genus (Applied Imaging, UK). In the first instance, cells were karyotyped and analysed by enhanced DAPI banding. Detailed paint analysis was then performed by assessing paint coverage for each individual fluor down the length of each individual chromosome, using both the raw and processed images for each fluor channel. A cell was classified as being apparently normal if all 46 chromosomes were observed by this process, and subsequently confirmed by the Genus m-FISH assignment, to have their appropriate combinatorial paint composition down their entire length.

Classification of Aberrations

For 3-colour FISH: When a painted chromosome was involved in an exchange, the painting pattern was classified according to the scheme of Savage and Simpson . In brief, any exchange that involved three or more breaks in two or more chromosomes was classified as Complex and those which involved a maximum of two breaks in two chromosomes were scored as Simple. Each complex aberration was determined to be transmissible or non-transmissible according to the presence of dicentrics or acentric fragments and all insertion-type rearrangements were noted. Simple reciprocal translocations and Simple dicentrics with acentric fragment were termed as 2B and 2A respectively, or as their associated incomplete types. Chromosome breaks and fragments not obviously involved in an exchange were classified as a Break only. Inversion events were not included in the scores due to high detection error. The occurrence of chromatid-type aberrations was assessed by DAPI staining during the 3-colour analysis procedure.

For m-FISH: Classification was as above with the difference that each Complex observed was assigned the most conservative C/A/B (minimum number of Chromosomes/Arms/Breaks) involved .

Statistical Analysis

Fisher's exact test (or its multi-sample equivalent) was employed to compare data that was expressed as a proportion of abnormal cells or as a ratio. The exact conditional binomial test (or its multi-sample equivalent) was used to analyse data when expressed as the mean number of aberrations per cell.

RESULTS

3-colour FISH

Table 1 details the exposure history and the chromosome exchange aberrations that were visualised by 3-colour FISH for subjects 3-6. All available metaphase cells for each subject were painted and all those cells of acceptable quality were analysed: 560, 366, 871 and 466 cells for subjects 3, 4, 5 and 6 respectively.

Complex chromosome aberrations were observed in all subjects analysed (3-6). The highest frequency observed was found in subject 4 who, as detailed in Table 1, received the highest dose (internal and external) in this study group. Statistical differences in complex frequency were seen between subjects 4 (0.0273) and 5 (0.0057) (p=0.0033) and

subjects 4 and 6 (0.0043) (p=0.0074) (Table 1). No differences were observed between subjects 3 and 4, 3 and 5, 3 and 6 or 5 and 6.

Each complex was classified according to its potential transmissibility. Consistent with on-going α-particle irradiation, we found non-transmissible type complex aberrations to be the dominant type of complex aberration in all of the Pu exposed but not externally exposed subject. Although we found no statistical difference in the frequency of visible insertions between the Pu subjects (3-5) and the externally exposed (subject 6), insertion events were seen to be common features of both transmissible and non-transmissible complexes in the Pu exposed (Table 1).

Overall it was simple exchanges, predominantly complete and incomplete reciprocal translocations (2B), which represented the major exchange type aberration observed in all subjects (3-6) (Table 1). Consequently, when this data was expressed either as the $simple_{total}$:$complex_{total}$ (S:C) ratio (2, 2.8, 2.4 and 4) or 2B:insertion (I) ratio (1.4, 4.5, 1.7 and 4) radiation quality signatures for subjects 3-6 respectively, no statistical differences were seen between any of the subjects .

A higher frequency of chromosome breaks apparently not involved in an exchange was seen for subject 4 (0.0219) compared to subjects 3 (0.0054) (p=0.031), 5 (0.0046) (p=0.0087) and 6 (0.0021) (p=0.013). No differences in frequency of chromatid-type aberrations was observed between any of the subjects studied (0.0054, 0.0, 0.0069 and 0.002 for subjects 3-6 respectively).

m-FISH Analysis

Table 2 details the chromosome aberrations that were visualised by m-FISH for subjects 3-6 and also, two regional control samples (subjects 1 and 2). All available metaphase cells for each subject were painted and all those cells of acceptable quality were analysed: 182, 151, 348, 130, 349 and 230 cells for subjects 1, 2, 3, 4, 5 and 6 respectively.

No complex aberrations were observed in either regional control. Simple (0.0066) and chromatid (0.0066) aberrations in subject 2 accounted for all the damaged cells observed (1.3%) and was consistent with background levels of damage. A far higher proportion of cells was observed to be damaged in subject 1 however (6.0%). This represented simple rearrangements (0.0165), chromatid breaks and gaps (0.0275), chromosome breaks not involved in an exchange (0.0165) and tetraploid cells (0.0055). Subsequent details on the exposure history of this individual have revealed current employment in the chemical industry, with no record of occupational exposure to radiation.

The frequency and classification of exchange aberrations detected by 3-colour FISH in subjects 3-6 can be directly compared with those aberrations visualised by m-FISH (Tables 1 and 2). As expected, the proportion of exchanges classed as complex is greater when the samples are assayed by m-FISH, resulting in reduced S:C (1.3, 1.1, 1.3 and 1.7) and 2B:insertion (I) (1.0, 1.1, 0.8 and 4.0) ratios to that previously seen, for subjects 3-6 respectively. No statistical difference between any of the exposed subjects for either of the radiation quality signatures tested was seen.

The frequency of complex aberrations observed in the Pu subjects (3-5) ranged from 0.0229 – 0.0846 (Table 2). To examine the complexity of each aberration in detail, we estimated the minimum number of Chromosomes/Arms/Breaks involved in each complex

and classified each in terms of completeness, transmissibility and number of visible insertions. Table 3 shows that the majority of all complex aberrations were 'complete' (C) in that all 'break-ends' had illegitimately rejoined. In some instances, the full rearrangement could not be visually resolved, particularly where break-ends were sub-telomeric, leading to the classification of these complex aberrations as unresolved-incomplete (UI), however in the main, these events could be assumed quite easily to also be complete . True-incompleteness (TI), where break-ends remained unrejoined, was rare . Based on this, we could be confident that the size of each complex scored was a good representation of the minimum complexity of each aberration. Complex sizes ranged from 2/2/3 to 4/5/6, 2/2/3 to 8/9/10 and 2/2/3 to 7/8/11 for subjects 3-5 respectively (Table 3). As expected, non-transmissible events represented the dominant complex aberration type and insertion-events were seen to be common features of both transmissible and non-transmissible types of rearrangement. A significant difference in the total frequency of visible insertions was seen between regional controls (1+2) and all of the Pu exposed ($p=0.0079$, $p=0.0086 \times 10^{-4}$, $p=0.0079$ for subjects 3-5 respectively). Similarly, a significant difference was seen between subjects 4 and 6 ($p=0.00011$), but not between controls (1+2) and subject 6 (Table 2). The overall frequency of insertions detected by m-FISH compared to 3-colour FISH increased 2 to 15 fold (Tables 1 and 2). Complex aberrations with more than 1 visible insertion were also observed. These generally involved different chromosomes and as such were not consistent with instability driven amplification events.

Table 4 shows the total damage observed in each cell that contained at least one complex exchange, as observed by DAPI counterstain only and classified as described by

Tawn . Abnormal monocentrics and fragments/rings represent the major base type of the complexes observed with multicentrics greater than dicentrics occurring rarely.

A significant excess of chromatid aberrations was observed in subject 4 (0.0615) compared to all other subjects (excluding subject 1 as discussed above) (Table 2). The majority of these chromatid aberrations were found in cells that contained no other chromosomal damage. This is illustrated by the low frequency of cells which contained a complex aberration in addition to an apparently separate simple exchange and/or a chromosome break and/or a chromatid gap/break (0.0057, 0.0153, 0.0 and 0.0 for subjects 3, 4, 5 and 6 respectively).

DISCUSSION

The identification of a cytogenetic biomarker of exposure to densely ionising high-LET α-particle radiation has recently focussed on the detection of insertions (transmissible complexes) and subtle intrachromosomal events. The principal reasons for this interest being 1) the increased resolution afforded by current cytogenetic techniques 2) the fact that these aberrations are mechanistically characteristic of α-particle exposure and 3) the expectation that these aberrations will be stable with time . By contrast, non-transmissible complex aberrations are considered to have limited relevance as radiation quality biomarkers and as a consequence are rarely reported in full . In this study, we found non-transmissible complexes to account for between 0.5 and 2.5% of the total complex frequency (0.6-2.7%) when observed by 3-colour FISH, in subjects chronically exposed to internal Pu over a period in excess of 30 years. This frequency increased when the

same samples were assayed using m-FISH (1.7-3.9%). In other words, non-transmissible complex aberrations represent the major complex type in individuals who are chronically exposed to high-LET α-particle radiation from internal deposits of Pu (2.3-8.5% total complex frequency).

Complexes and cells containing unstable aberrations have been observed elsewhere in radiation workers, including those known to have internal deposition of Pu and in thorotrast patients, many years after exposure . We show here however that unless all of the chromosomes are uniquely painted, then not only is there a reduced frequency of detection of complex aberrations and corresponding stable insertions, but there is also a loss in appreciation of the total cellular damage (Tables 1 and 2) . To expand on this, of the total exchange aberrations observed by 3-colour FISH, simple exchanges dominated in the Pu exposed individuals 2-3 fold over complex aberrations and large fractions of these simples were classified as incomplete . This proportion became closer to 1:1 when the same subjects were analysed by m-FISH whereupon the majority of the simples were resolved as complete reciprocal translocations. This demonstrates that after high-LET exposures, incomplete simples, detected using a non-24-colour FISH technique, are most likely to be unresolved complexes . Interestingly, the scale of the difference in complex frequency obtained by the two methods should also be considered when converting translocation data into the full genome frequency .

m-FISH enables genome-wide interchromosomal damage to be completely resolved, excluding subtle rearrangements between homologues, intrachromosomal-events and cryptic interchromosomal rearrangements below ~ 11 MB . What this allows is the discrimination of whether all the damaged chromosomes in each damaged cell are part of

the same rearrangement or whether there are multiple apparently independent exchanges present . Qualitatively this is quite informative. For example, if 4 different chromosomes are damaged in a lymphocyte, mis-repair is more likely to result in the formation of 2 independent simples if the cell had been exposed to low-LET X-rays, but as 1 complex involving all 4 chromosomes, if the damage was induced by the nuclear traversal of a single high-LET α-particle . Earlier, we demonstrated that the size of each complex observed by m-FISH (based on number of chromosomes involved) *in vitro* was consistent with the number of chromosome domains predicted to have been traversed (between 1 and 8 different domains in a G_0 lymphocyte). Thus, the spectrum of total cellular damage and size range of complex aberration induced appears to be quite specific to the initial α-particle nuclear traversal .

The results of the present *in vivo* study indicate that the total cellular damage in the Pu exposed subjects and the size and transmissibility of each complex detected by m-FISH was consistent with those observed *in vitro* after exposure to a mean of 1 high-LET α-particle per cell. This suggests that the complex aberrations observed in these individuals were directly induced by α-particle exposure. The Pu body burdens' of the subjects studied here are however extremely large and all of the individuals are in poor health meaning that you cannot exclude the possibility that the aberrations observed are a consequence of other indirect effects either of the exposure itself or of disease-induced inflammation . Indeed, cytogenetic aberrations were only detected in monkeys exposed to soluble and insoluble forms of inhaled Pu, when the exposures were high and histological changes to the lung were observed . In contrast to this, numerous human cytogenetic studies show evidence for elevated frequencies of aberrations in PBL at lower doses not

associated with radiation disease . In addition, there is no evidence for bystander-induced or instability-driven mechanisms to characteristically produce chromosome aberrations of the complexity observed here, both in terms of the complex aberrations themselves or as the genome-wide damage per cell. Both of these phenomena are documented to produce chromatid-type aberrations and simple chromosome rearrangements but not complex exchanges . Based on the above considerations it is therefore likely that the complex aberrations observed do represent damage directly induced by the α-particle traversal. Further, since the lowest dose a cell can receive is that from a single α-particle then any decrease in dose will only relate to a decrease in the number of cells 'hit' by an α-particle and will have no impact on the complexity of aberration observed in each damaged cell.

The detection and exploitation of non-transmissible complex aberrations, in addition to transmissible complexes and insertions, as biomarkers of high-LET α-particle exposure could therefore be relevant for the discrimination of radiation quality exposure. However in this study, complexes were also found in subject 6 who had no record of Pu exposure (Tables 1 and 2). To examine this finding in more detail we compared the aberration frequency observed by both 3-colour (converted to full genome frequency) and m-FISH with that which would be expected assuming the recorded chronic exposure of 15 cSv external radiation (Table 5A). We found that the frequency of both simple and complex aberrations observed in subject 6 was higher than would be expected and have estimated that the external dose required to induce the frequencies observed would be in the order of ~ 1 Sv (Table 5B). There was no evidence of clonal expansion of stable translocations which could account for this large discrepancy between recorded dose and aberration frequency and although the actual number of cells analysed for this subject is relatively

low, it would appear that either the dose estimates are incorrect or subject 6 has also been exposed to high-LET radiation.

As mentioned above, according to *in vitro* studies, we would expect complex aberrations to be induced in the majority of cells directly traversed by high-LET α-particles . For the Pu subjects examined in this study however, simple reciprocal translocations represented the dominant exchange type observed, even when assayed by m-FISH. Again, we saw no evidence of clonality which could account for this high simple frequency, which was in excess to expectation both based on age and estimated external exposures (Table 1) . Based on the finding that the majority of these simples were of the stable (2B) type, it was assumed that they were not recently produced by on-going Pu exposure, either from the α-particle itself or from γ-rays emitted during α-particle decay, due to the expectation that simple dicentrics (2A) should be formed with equal likelihood . Therefore, by what mechanism were these simples induced? Assuming the dose estimates in Table 1 are reliable, then the elevated translocation frequencies observed could represent the occurrence of on-going genomic instability . Alternatively, it is possible that α-particle-induced damage in primitive stem cells of the haemopoietic compartment predominantly results in the formation of simple exchanges. The stable types of those initially-induced aberrations would then repopulate the peripheral lymphocyte pool. However, stem cells, like lymphocytes, are spherical in shape, meaning that each α-particle will have the same likelihood of intersecting 1,2,3,etc chromosome domains as predicted for the lymphocyte . Since we have proposed that α-particle-induced complexes may be formed by the sequential linking of mis-repair events from the multiple domains traversed by the α-particle, then we would expect non-transmissible

complex aberrations to be the predominant type of aberration formed in a hierarchical stem cell. A small proportion of cells would be expected to contain directly induced stable simple exchanges that most likely formed as a consequence of an α-particle skimming the edge of the nucleus and intersecting 1 or 2 chromosome domains. Such cells, together with those cells that only contain transmissible-type complex aberrations, would be capable of maturing and repopulating the peripheral circulatory pool.

It is therefore possible that the high frequency of simple aberrations observed in the Pu subjects represent the stable progeny of stem cells directly damaged by α-particle exposure in the bone marrow . Consequently, if these arguments are valid, then the usefulness of radiation quality signatures, which predict low S:C or low 2B:insertion (I) ratios for high-LET exposures, is extremely limited . A biomarker of exposure to high-LET α-particles, when assayed by 24-colour karyotyping, would then rely solely on the detection of cells which contain complex aberrations. Non-transmissible and transmissible complex aberrations would be suggestive of recent and on-going α-particle exposure of mature lymphocytes in the peripheral circulation . In addition, a proportion of the transmissible complex aberrations that are observed would represent surviving cells exposed in the bone marrow compartment . Evidence that complex aberrations are induced in stem cells is shown by the relatively high frequency of transmissible complexes in subject 4, and the finding of a clonal transmissible complex in the same subject (Table 2). In addition, Littlefield *et al*., observed transmissible complexes in lymphocytes of a thorotrast patient and concluded they were the progeny of irradiated stem cells. In the same study, Littlefield *et al*., also proposed that highly complex aberrations could be indicative of exposure to densely ionising radiation .

In conclusion, complexity and non-transmissibility appear to be the major cytogenetic feature of α-particle induced chromosomal aberrations. Differing staining and painting techniques, depending on their resolution, will reveal different elements of the total damage in the cell. The likelihood that even quite subtle (apparently) transmissible damage, assuming it is directly induced, is part of a larger complex event that will ultimately be lethal to the cell, is therefore high. However as demonstrated here and elsewhere, it is important to note that heavily damaged cells appear to be quite long lived *in vivo* such that significant proportions of non-transmissible complex aberrations can be detected after decades of chronic, albeit high dose, exposure . Cell life-span and immunological status will clearly influence the frequency of detection of any chromosome aberration, but for chronic exposures, complex chromosome aberrations that are indicators of α-particle exposure will continually arise (see Figure 1).

We have shown here the potential usefulness of exploiting complex aberrations as a biomarker of exposure to high-LET α-particles and the dependence of this biomarker on 24-colour karyotyping. Further studies are needed to fully determine the specificity of this biomarker of exposure, to high-LET α-particles and its utility at lower levels of exposure that may be more relevant to the general population.

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REFERENCES

LEGEND

Figure 1.

m-FISH metaphase (A) and karyotype (B) of a complex rearrangement (7/8/11) observed in Pu exposed subject. Chromosomes 2,6,6,7,8,12 and 17 are involved. The definition of a α-particle-induced complex, observed by m-FISH in a lymphocyte cell, is consistent with the following. Each complex would be expected to involve up to 8 different chromosomes with on average 1-2 misrepaired breaks down the entire length of each chromosome involved. Insertion events would be visible regardless of the stability of the complex. Unstable complexes that have successfully reached second mitosis are not expected to persist further and will have numerous acentric losses and/or duplications, however the initially-induced α-particle-induced complex can virtually always be deduced from the aberration observed . In the main, all of the damaged chromosomes present in a cell would be associated in the same complex event when visualised by m-

FISH. However, when broken down into component base types as seen by DAPI, the same damage would be visualised as consisting of an excess of abnormal monocentrics and fragments (Table 4). Cells containing two apparently independent complexes, consistent with that cell being traversed by two separate α-particle tracks and cells which contain a complex in addition to simples or breaks may also be observed.

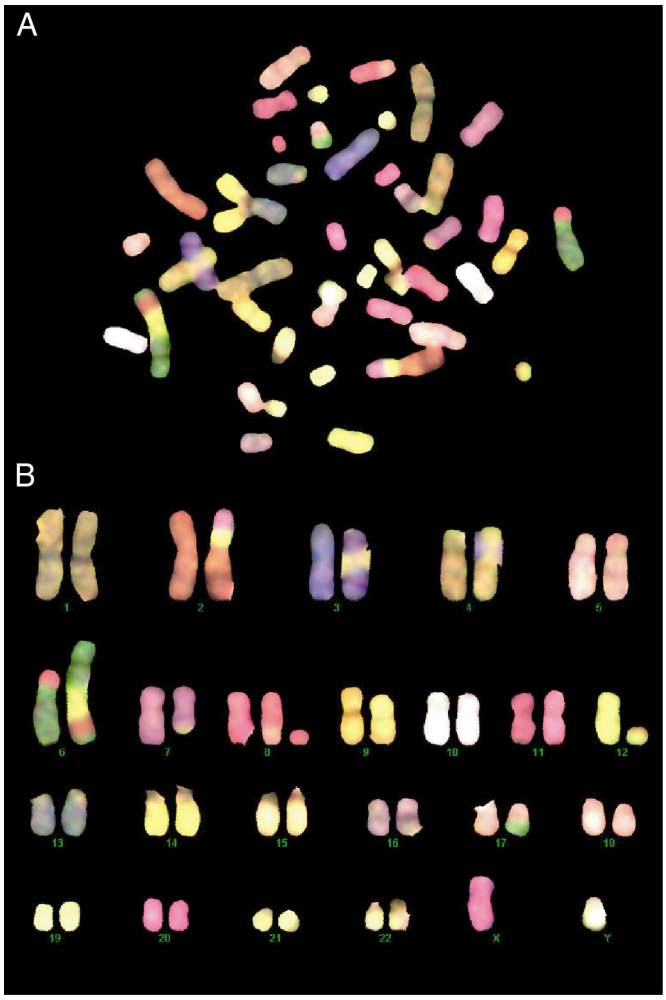

Figure 1

TABLE 1

Chromosome Exchange Aberrations Visualised by Three-Colour FISH

	Subject			
	3	4	5	6
Age at blood sampling (yrs)	55	55	60	63
Working period	1963-96	1963-73	1979-86	-
External dose (cSv)	11.8	31.7	14.2	~15
Plutonium activity (kBq)	2.59 (medium)	11.10 (high)	2.22 (medium)	- (low)
Total cells	560	366	871	466
% Damaged	5.2	10.4	2.9	2.1
Simple (frequency)				
2B	0.0179	0.0246	0.0057	0.0086
Incomplete 2B	0.0107	0.0355	0.0034	0.0064
2A	0.0	0.0055	0.0012	0.0022
Incomplete 2A	0.0036	0.0109	0.0034	0.0
Total	**0.0321±0.0076**	**0.0765±0.045**	**0.0138±0.004**	**0.0172±0.0061**
Complex (frequency)				
Transmissible	0.0054	0.0027	0.0012	0.0043
Non-transmissible	0.0107	0.0246	0.0046	0.0
Visible insertion	0.0125	0.0055	0.0034	0.0022
Total	**0.0161±0.0054**	**0.0273±0.0086**	**0.0057±0.0026**	**0.0043±0.003**

TABLE 2

Chromosome Aberrations Visualised by m-FISH

	Subject					
	1	2	3	4	5	6
Total Cells	182	151	348	130	349	230
% Damaged	6.0	1.3	8.3	20.0	7.2	8.3
	Frequency					
Simple						
2B	0.0110	0.0066	0.0230	0.0923	0.0172	0.0174
Incomplete 2B	0.0	0.0	0.0086	0.0	0.0057	0.0044
2A	0.0	0.0	0.0058	0.0	0.0029	0.0
Incomplete 2A	0.0055	0.0	0.0029	0.0077	0.0029	0.0
Total	**0.0165±0.0095**	**0.0066±0.0066**	**0.0402±0.0108**	**0.1000±0.0277**	**0.0287±0.0091**	**0.0217±0.0097**
Complex						
Transmissible	0.0	0.0	0.0058	0.0462	0.0057	0.0044
Non-transmissible	0.0	0.0	0.0259	0.0385	0.0172	0.0087
Visible insertion	0.0	0.0	0.0230	0.0846	0.0229	0.0044
Total	**0.0**	**0.0**	**0.0316±0.0095**	**0.0846±0.0255** [a]	**0.0229±0.0081**	**0.0130±0.0075**
One complex/cell	-	-	0.0287	0.0846	0.0200	0.0130
> One complex/cell	-	-	0.0029	0.0077	0.0029	0.0
Chromosome breaks	0.0165	0.0	0.0172	0.0	0.0057	0.0130
Chromatid break/gaps	0.0275	0.0066	0.0144	0.0615	0.0143	0.0130
Tetra/aneuploid cells	0.0055	0.0	0.0029	0.0	0.0115	0.0217

[a]Clonal transmissible complex found in two cells therefore counted as one event for total complex frequency

TABLE 3

Complex Aberrations Visualised by m-FISH

Subject	Complex	Complex size	Completeness	Transmissible	# Visible insertions
3	1	2/3/4	C	N	1
	2	2/2/4	C	N	1
	3	3/4/4	C	N	0
	4	4/5/6	C	N	2
	5	4/5/5	UI	N	0
	6	2/2/3	C	Y	1
	7	3/3/4	TI	N	0
	8	3/3/4	C	Y	1
	9	3/3/3	UI	N	0
	10	3/5/5	C	N	2
	11	3/3/3	UI	N	0
4	1	3/3/3	C	Y	0
	2	2/2/3	C	Y	1
	3	6/7/9	UI	N	3
	4	3/4/5	C	N	1
	5	3/3/3	C	Y	0
	6	8/9/10	UI	N	2
	7	3/4/4	C	N	1
	8	3/3/3	C	Y	0
	9	3/3/3	C	Y	0
	10	3/5/5	C	Y	2
	11	6/6/6	UI	N	0
	12	4/4/5	C	Y	1
5	1	3/3/3	UI	N	0
	2	3/3/3	C	Y	0
	3	6/6/6	C	N	0
	4	7/8/11	C	N	3
	5	2/2/3	C	Y	1
	6	3/3/5	C	N	2
	7	4/4/7	UI	N	1
	8	3/4/5	C	N	1
6	1	3/3/4	UI	N	1
	2	3/3/3	C	Y	0
	3	3/3/3	C	N	0

TABLE 4

Total Damage in Cells Containing A Complex Visualised By DAPI

Subject	Cell	Complex	Total number in cell				
			Abnormal monocentric	Dicentric	Tricentric	Acentric ring + centric ring	Acentric fragment
3	1	1	2	1	-	1	3
	2	2	1	-	-	1	1
	3	3	-	2	-	1	5
	4	4	2	1	-	-	1
	5	5	3	-	-	-	2
	6	6	2	-	-	-	-
	7	7	1	1	-	1	2
	8	8	3	-	-	-	-
	9	9	2	-	-	-	-
	10	10+11	1	1	1	-	3
4	1	1	3	-	-	-	-
	2	2	4	-	-	-	-
	3	3	4	1	-	-	-
	4	4	1	1	-	-	2
	5	5	3	1	-	-	-
	6	6+7	4	3	-	-	3
	7	8	3	-	-	-	-
	8	9	3	-	-	-	-
	9	10	3	-	-	-	-
	10	11	4	1	-	-	1
	11	12	4	-	-	-	-
5	1	1	1	-	-	-	-
	2	2	3	-	-	-	-
	3	3	2	2	-	-	2
	4	4	5	1	-	1	1
	5	5	2	-	-	-	-
	6	6+7	3	2	-	-	3
	7	8	1	1	-	1	1
6	1	1	2	-	-	-	1
	2	2	3	-	-	-	-
	3	3	1	1	-	-	1

TABLE 5

Dose Considerations for Subject 6

A. If external exposure ~ 15 cSv then,

	Expected	Observed	
Frequency		3-colour FISH (F_G frequency)	M-FISH
Simple	0.0084 [a]	0.0243 [b] 0.0425 [c]	0.022
Complex	0.0014 [d]	-	0.013

[a] Expected F_G frequency according to Lucas *et al*., (1992) .
[b] F_G frequency if only complete translocations (2B) used in conversion.
[c] F_G frequency if both complete (2B) and incomplete translocations used in conversion
Note: Single paint formula used throughout since no corresponding formula for chromosomes painted with three different colours plus DAPI available. This will marginally over-estimate the F_G frequency because exchanges between painted chromosomes are included in the scoring.
[d] Expected frequency of complex aberrations detected by m-FISH according to Loucas and Cornforth (2001) .

B. Estimation of external dose

Translocations observed by 3-colour FISH	F_G	Dose (cSv) [c]	Expected frequency of complex aberrations [d]
2B	0.0243 [a]	62	0.0118
2B + incomplete 2B	0.0425 [b]	123	0.0327

[a] F_G frequency if only complete translocations (2B) used in conversion.
[b] F_G frequency if both complete (2B) and incomplete translocations used in conversion.
[c] Doses estimated from aberration frequency, using dose reconstruction parameters of Knehr and Bauchinger (2000) . Since low dose rate exposure is assumed, only linear slope (α=3.2±0.7) is used.
[d] Expected frequency of complex aberrations detected by m-FISH corresponding to reconstructed dose, according to Loucas and Cornforth (2001) .