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Effect of Low Frequency Electromagnetic Fields (LF-EMFs) on *c-myc* Oncogene Expression Level in Peripheral Blood Mononuclear Cells (PBMCs)

Yehia Mustafa[1], Sanaa Riad[1], Salah Abdel-Rahman[2*], Maha El-Demellawy[3] and Sarah El-Messeiry[1]

[1]Department of Genetics, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.
[2]Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.
[3]Department of Medical Biotechnology, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors YM, SR and SE designed the study, wrote the protocol, performed the statistical analysis and calibrated and settled exposure unit. Author SA performed quantitative real time polymerase chain reaction (qRT–PCR), analyzed qRT-PCR results and prepared the final manuscript. Author ME managed cell culturing (PBMCs and HELA-S3) and optimized PBMCs viability, morphology and count. All authors read and approved the final manuscript.

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**Corresponding author: Email: salahmaa@yahoo.com;*

ABSTRACT

Low frequency electromagnetic fields (LF-EMFs) which can be generated from homes and workplaces appliances can lead to alteration in oncogenes causing cancer diseases such as leukemia, nervous system tumors, lymphoma and breast cancer. To investigate the effect of LF-EMFs on *c-myc* oncogene expression level, primary cell culture of human peripheral blood mononuclear cells (PBMCs) were exposed to AC (50 Hz) electromagnetic flux density: 0.37mT, 0.82mT, 1.22mT, 1.68mT, 2.1mT, 2.47mT, 2.85mT, 3.33mT, 3.72mT, 3.92mT, 4.32mT and 4.67mT using exposure unit. After four days of exposure, when initial changes in cell viability, morphology and count between exposed and unexposed cells were noted, total RNA was extracted to evaluate the expression level of *c-myc* oncogene by quantitative real time PCR (qRT-PCR). The results showed that *c-myc* oncogene expression level began to increase gradually from value 1.69% at 0.82mT reaching to the maximum expression level value 4.65% at 4.67mT. This result refers to an increasing in LF-EMFs exposure offset by an increasing in *c-myc* oncogene expression level, affecting different cellular functions. This study indicates that LF-EMF is environmental pollution effects on the expression level of oncogenes which increase the risk of human cancer diseases.

Keywords: PBMCs; HELA-S3; LF-EMFs; c-myc gene; qRT-PCR.

1. INTRODUCTION

Natural environment has changed with introduction of a vast and growing spectrum of human-made electromagnetic fields (EMFs) caused by a variety of home and workplaces appliances. It has previously been proposed that these fields were initially considered too weak to interact with human biological systems and incapable of affecting genetic material [1]. There are three main types of low frequency electromagnetic fields (LF-EMFs) exposure; indoors, outdoors and occupational. Indoors: Normal power line wiring with exposure range 0.03mT, Bad wiring in homes with exposure range 0.2-3mT [2], Hair dryer at 3 cm distance with exposure range up to 2.0mT (Federal Office for Radiation Safety) and Electric shaver at 3 cm distance with exposure range up to 1.5mT [3]. Outdoors: Transportation with exposure range less than 0.03mT and High voltage power line stations with exposure range greater than 8mT. Occupational: Telecommunications (telephone linemen, technicians and engineers mean) with exposure range 0.35-0.43mT [4], In an office environment, magnetic fields are generally except near computers, photocopiers or other electronic equipment with exposure range at or below 0.17mT [5], Railway workers with exposure range 4mT, Electricians with exposure range 1.4-3.6mT and Forestry and logging workers with exposure range 2.48mT [4]. The first report in this direction was that about the potential harmful effects of power lines, associated with residential, rather than with occupational exposure [6]. In the early 1990s, the Wertheimer and Leeper study was repeated in various locations and enormous amounts of studies were published on human cancer diseases caused by electromagnetic fields. However, cancer is caused by alterations in oncogenes (carcenogenes). These alterations are usually somatic events, although germ-line mutations can predispose a person to heritable or familial cancer [7]. *c-myc* gene is oncogene located in human chromosome 8 and believed to regulate global chromatin structure [8]. Mutated version of this oncogene is found in many cancers, causing its over-expression. This leads to the unregulated expression of many genes, some of which are involved in cell proliferation and results in the formation of cancer. The aim of the current study is to investigate the effect

of LF-EMFs (50 Hz) which can be generated from homes and workplaces appliances on the expression level of the *c-myc* oncogene in human primary cell culture (PBMCs).

2. MATERIALS AND METHODS

2.1 Cell Cultivation

2.1.1 PBMCs

Veins blood was collected from healthy female volunteer (24 years old) into vacationers heparinized tube. Lymphocytes were isolated from whole blood using Ficoll-Paque density gradients as known. Cells suspended in culture RPMI 1640 supplemented with 10% fetal bovine serum (FBS). Cells were divided into 25 cm^2 cell culture flasks with about 0.5×10^6 cells in 2.5ml of pre–filtered RPMI 1640 supplemented with 2mM L-glutamine and 1M HEPES buffer. Then, the cells were exposed for specific time to specific doses of LF- EMFs. After exposure, cells were incubated at 37°C humidified 5% CO_2 until the suitable time for total RNAs extraction and cells count and viability determination. PBMCs count and viability were determined using Trypan-blue exclusion test by mixing 30µl of cell suspension and 30µl Trypan-blue. The mixed cells were added to hemocytometer counting slide and counted under 1000X phase contrast microscope. Where, the dead cells appeared with blue color, while the live cells were transparent.

2.1.2 HELA-S3 cells

HELA-S3 cells were obtained from ATCC and used as cancer positive control. 10ml pre-warmed pre-filtered Ham's F12 culture media supplemented with 2mM L-glutamine; 1M HEPES buffer and 20% fetal bovine serum (FBS) were added to cells and centrifuged at 1200 rpm for 10 min for washing and removing any excess freezing media. Cell pellets were re-suspended in 75 cm^2 cell culture flask using Ham's F12 culture media then incubated in CO_2 incubator at 37°C humidified, 5% CO_2. After 2 days, the cells were examined under 2000X phase contrast microscope. After formation of adherent cell layer, non-adherent cells were removed by discarding the media from the flask. The detached cells were removed in 50 ml falcon tube and centrifuged at 1200rpm for 5 minutes, supernatant was discard and the cell pellet was transferred into a new 75cm^2 cell culture flask. Finally, the cells were ready for further analysis.

2.2 LF-EMFs Exposure System Design

Primary cell cultures of human peripheral blood mononuclear cells (PBMCs) were in vitro-exposed once for one hour to alternating current (AC; 50 Hz) electromagnetic flux density: 0.37mT, 0.82mT, 1.22mT, 1.68mT, 2.1mT, 2.47mT, 2.85mT, 3.33mT, 3.72mT, 3.92mT, 4.32mT and 4.67mT (appliances's EMFs range) using exposure unit designed specifically for this study. Electromagnetic fields (EMFs) are typically generated by alternating current in electrical conductors. The simulation of LF-EMF exposure was performed in an incubator designed specifically for the cells exposure (Fig. 1). The source of LF-EMF was established by powerful wave generator which produces electromagnetic field in the range from 10 to 500 cps (cycle per second). The flux density of the field was measured by Hand-Held Gauss/Tesla Meter and controlled by adjusting the Volt (12 V) and Ampere (4 A) in the SF-9584 *low Voltage* AC/DC power supply.

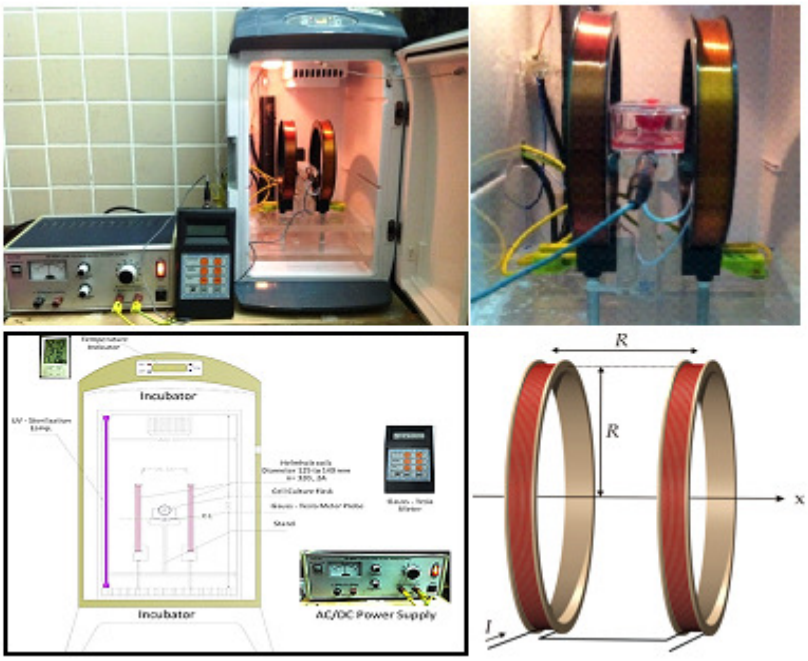

Fig. 1. Low frequency electromagnetic fields (LF-EMFs) exposure unit. This unit was designed specifically for this study. Up left, an incubator designed specifically for the LF-EMFs cells exposure unit. Up right, a clear vision (zoomed photo) of cells exposing process

2.3 Quantitative Real Time Polymerase Chain Reaction (qRT–PCR)

2.3.1 RNAs extraction

On the 4-th day of exposure, when initial changes in cell count and viability and morphology, total RNAs were collected from cells (PBMCs) using Gene JET RNA Purification Kit (Thermo Scientific, USA). In order to make sure that RNAs isolation process were successful, total RNAs samples were separated on 1.1% agarose gel electrophoresis. The extracted RNAs from different samples were quantified and qualified (purity) using NanoDrop Spectrophotometer. Finally, total RNAs samples were normalized (same concentration) to avoid any false increase in gene expression levels.

2.3.2 *C-myc* gene expression

Using SYBER Green 1-step qRT-PCR Kit (Thermo Scientific, USA), gene expression of *c-myc* (targeting gene) and *β-actin* (reference gene) was quantified by Real-Time PCR System (Thermo Scientific PikoReal) with the use of specific primers sequences (Forward/Reverse) 5′-CTTCTCTCCGTCCTCGGATTCT-3′/5′-GAAGGTGATCCAGACTCTGACCTT-3′ for *c-myc* gene and (5′-CGCGAGAAGATGACCCAGAT-3′/5′-GATAGCACAGCCTGGATAGCAAC-3′ for *β-actin* gene [9]. qRT-PCR was performed in a reaction mixture of 10µl using 0.1µl verso enzyme mix, 5µl 1-step QPCR SYBER mix (1X), 0.5µl RT-enhancer, 0.7µl forward and reverse primers (10pm), 1-2.6µl water (PCR grade) and 0.4-2.0µl RNA template (10ng). qRT-PCR program was applied as one cycle of cDNA synthesis at 50℃ for 15 minutes, one

cycle of Thermo-start enzyme activation at 95°C for 15 minutes and followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 65°C for 1 minute and extension at 72°C for 30 seconds.

3. RESULTS

3.1 Optimization of PBMCs Viability, Morphology and Count

PBMCs were isolated and divided into unexposed (control) and exposed cells (treated probe) with exposure range from 0.37mT to 4.67mT flux density (12 V and 4 A) with exposure duration of one hour. Also, by 2000 X phase-contrast microscope, we got a glimpse, when the changes of cell viability, morphology and count of the exposed cells had happened after four days of incubation, in comparison with the unexposed. Dramatic decrease in the count of exposed cells was observed in comparison of that of the unexposed, with average 1.133 million cells/1ml, while, exposed cells number was semi-constant with average 1.367 million cells/1ml (Fig. 2). Unexposed cells have an average lifespan until 4 days and then they begun to die, which could explain that the decrease in cells number after day 4, while, exposed cells have an average lifespan until 10 days, indicating some mortality features in response to the LF-EMFs exposure (Fig. 3). However, life time of PBMCs was elongated from 4 days to more than 10 days due to LF-EMFs exposure. Hence, the LF-EMFs exposure wasn't effective on PBMCs cells count within range from 0.82mT to 2.1mT flux density. However, PBMCs count was significantly increased within range from 2.47mT to 3.72mT, that's mean changes in cell cycle and mitotic division in exposed cells had happened (Fig. 4).

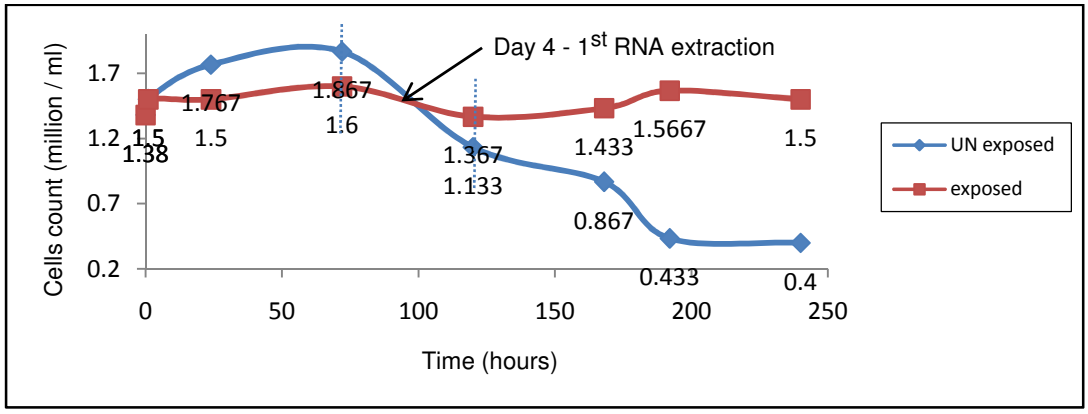

Fig. 2. Graph shows the growth curve for both exposed and unexposed cells in day 4 after LF-EMF's exposure. Where, unexposed cells count gradually decreased until 1.133 million cells/1ml in the fourth day and continued to decrease, while, exposed cells number was semi-constant with average 1.367 million cells/1ml

3.2 *C-myc* Expression Level

As it was mentioned previously, the right time of RNA extraction from cells after exposure to LF-EMFs was selected by results analysis of cells viability, morphology and count between exposed and unexposed cells in day 4. Due to the low frequency electromagnetic fields (LF-EMFs) exposure doses ranged from 0.37mT to 4.67mT, *c-myc* gene expression level

changes in primary cultures of human PBMCs. However, where the expression level of *c-myc* gene increased from 1.69 % at 0.82mT exposed cells reaching to the maximum value 4.65 % at 4.67mT exposed cells, affecting that different cellular functions by *c-myc* over expression (Fig. 5).

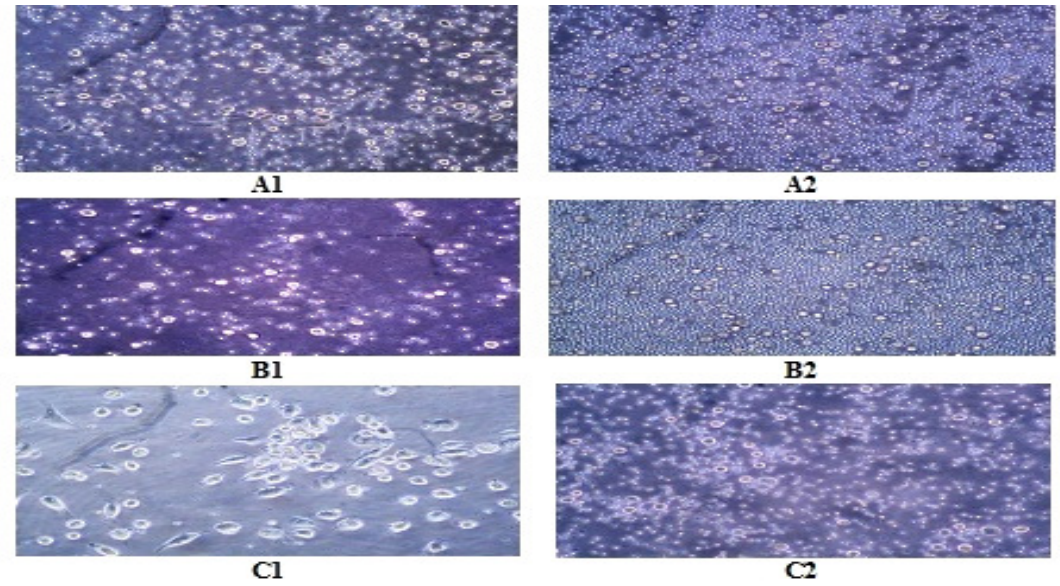

Fig. 3. Photomicrographs (under 2000X phase contrast microscope) show the differences in cells number between unexposed and exposed cells to LF-EMF's exposure. Where, A1/A2 is unexposed/exposed cells in day 4. B1/B2 is unexposed/exposed cells in day 8. C1/C2 is unexposed/exposed cells in day 12

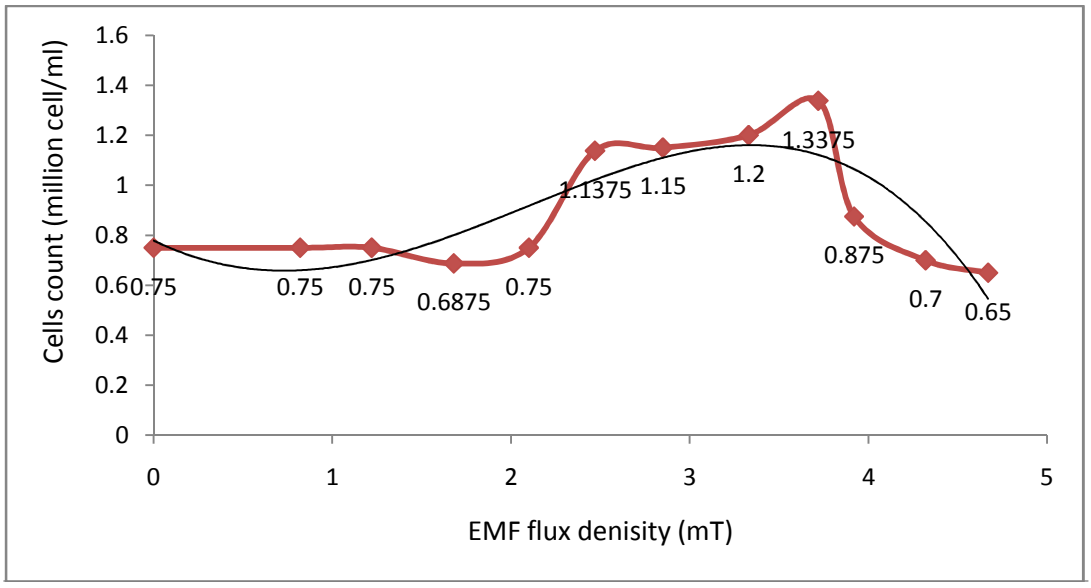

Fig. 4. Graph shows the relationship between EMFs flux density (mT) and cells count in the 1st duplicate and 2nd in day 4 after exposure

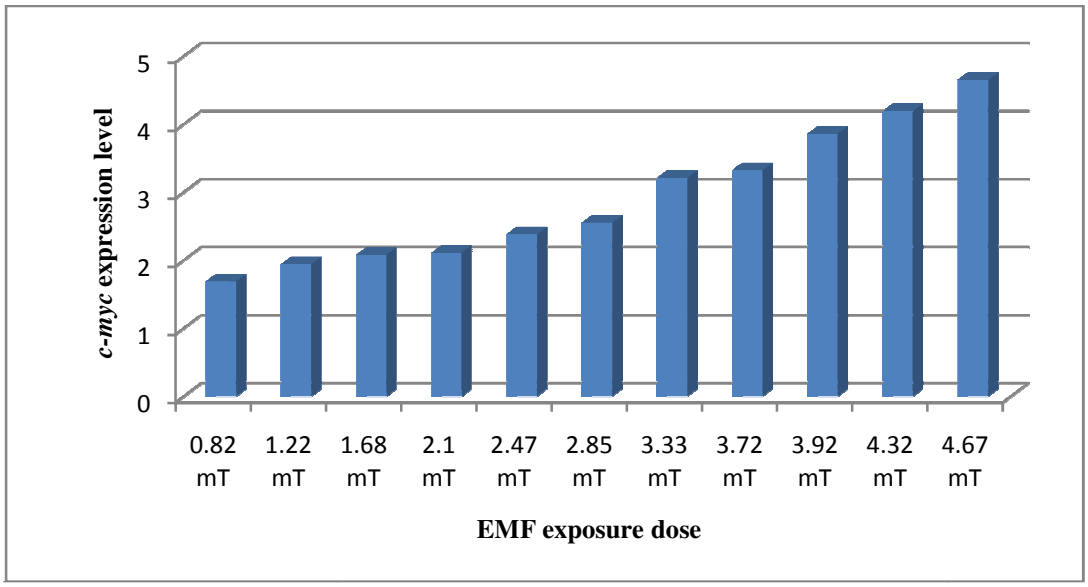

Fig. 5. The expression level of *c-myc* oncogene in exposed PBMCs (%) compared with unexposed cells in day 4 after exposure to AC electromagnetic flux density range from 0.82mT to 4.67mT (milliTesla)

4. DISCUSSION

Numerous studies have been carried in both *in vivo* and *in vitro* on bacteria, mice and human to know the effect of low frequency electromagnetic fields (LF-EMFs) on the genetic material, gene expression and other cell functions. The first study of the potential harmful effects of power lines associated with residential rather than occupational exposure was reported by [6]. The authors found that an increased incidence of childhood leukemia, lymphoma and nervous system tumors were associated with residential exposure to power line-frequency fields in Denver Colorado. In the early 1990s, Wertheimer and Leeper's study was repeated in various locations and a lot of studies were published on childhood previous cancers. Some studies confirmed Wertheimer and Leeper's results [10-18], while others found no effects [19-22].

In our current study, the effect of LF-EMFs (50 Hz) was investigated on the expression level of the *c-myc* oncogene which is known to be involved in normal cell proliferation and possibly also in tumor malignancy process in primary cultures of human PBMCs. The presented results clearly illustrated that there is a significant effect of low frequency electromagnetic fields (LF-EMFs) on cells functions and *c-myc* oncogene expression level. Those effects could be different according to the exposure flux densities which were very variable within range from 0.37mT to 4.67mT. LF-EMFs exposure wasn't effective on the PBMCs count within range from 0.82mT to 2.1mT flux density. PBMCs cells count have significant increasing within range from 2.47mT to 3.72mT in day 4 after exposures, which could be accepted as a proof for eventual changes in cell cycle and mitotic division in exposed cells. Also in day 4 after LF-EMFs exposure, *c-myc* gene expression level increased from 1.69% at 0.82mT exposed cells reaching to the maximum value 4.65% at 4.67mT exposed cells, affecting different cellular functions by *c-myc* over-expression which is known to be associated with. Over expression of *c-myc* gene associated with a variety of

hematopoietic tumors, leukemias and lymphomas, including Burkitt lymphoma which increases the capability of cells turning into cancer cells. We noticed that, in increase of LF-EMFs density, the expression level *c-myc* oncogene also increases, but not that great increasing turning cells into cancer cells such as HeLa cells, which have shown 59% increase in the expression level of the same gene. These results join us to the studies which confirmed the literature data [6]. Hence, the current study might be considered as the first and the best *in vitro* model for evaluation of LF-EMFs exposure on genetic materials of mammalian cells.

5. CONCLUSION

Without doubt, our bodies are exposed daily to enormous amount of electromagnetic fields (EMFs) in everywhere: outdoors, indoors and in workplaces. EMFs consider too weak to interact with human biological systems in the short term, but in the long term they have accumulative effects which could lead to different damages in the human genetic materials, causing dangerous diseases such as cancers. In general, our results indicated clearly that the increased exposure to low frequency electromagnetic fields (LF-EMFs) led to increasing in the viability, numbers and life time in exposed cells, in addition to increasing in the expression level of oncogenes. However, in primary cultures of human PBMCs, increase in the expression level value of *c-myc* oncogene increased from 1.69% at 0.82mT to 4.65% at 4.67mT expression level value after the fourth day of exposure. Hence, the performed study deserves attention, because the human body is exposed on increasing and continuously enormous amounts of low electromagnetic fields day after day and all these factors increase the risk of malignancies arising.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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