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MICE AGE IN CANCER RESEARCH: OVERLOOKED IMPLICATIONS FOR CLINICAL TRANSLATION

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SUMMARY

Mice are important study tools in biomedical research. The in vitro drug/compound anti-cancer findings usually need to be validated in preclinical models especially mice before moving into phase 1 human clinical trials. Cancer is still regarded as an old age disease but in many anti-cancer preclinical research, young mice aged between 6-8 weeks are used which is equivalent to the human age of 10-15 years old. Young mice may not adequately replicate the physiological condition of average human cancer patients; hence preclinical trial outcomes may be too hopeful. As such, although expensive, it is very important to design preclinical experiments by including 60 weeks old geriatric mice which is equivalent to approximately 50 years old cancer patients because older mice more accurately mimic the actual cancer patients.

KEYWORDS: Age, Cancer research, Human clinical trials, Mice, Preclinical studies

INTRODUCTION

The risk of getting cancer increases as one gets older. Cancer may affect anyone of any age; however, it mostly occurs in those who are 50 years old or older. The onset of cancer occurs when our cells undergo damage which cannot be fixed by repair pathways. As we age, the accumulation of cell damage increases, increasing the risk of developing cancer (Cancer Research UK 2023). In the United States, just 1.7% of all cancer-related fatalities occur before the age of 40, while 90% of malignancies are diagnosed in those over the age of 50. Cancer risk increases with age due to accumulated genetic mutations, altered cellular environments, and weakened immune responses (Laconi et al. 2020). Ageing cells lose some of their functionality and fitness, creating a vulnerable environment. Cancer cells exploit this situation by becoming more functional and fitter, allowing them to outperform ageing cells, thus driving tumour progression (Sedrak and Cohen 2023). This trend is seen in most common malignancies with the median age of a cancer diagnosis being 66 years. For breast, colon, lung, and prostate cancers, the median age at diagnosis is 62, 67, 71, and 66 years, respectively (Laconi et al. 2021). This editorial aims to highlight the critical role of the age of mice used in preclinical cancer studies and their significant implications for the translational success of these studies.

Immuno-senescence is characterised by the gradual deterioration of the immune system and is associated with ageing affecting cancer development and treatment efficacy (Weyand and Goronzy 2016). For instance, the T cell response has significant age-related alterations that will eventually impair its primary function (Laconi et al. 2021). The Lancet Healthy Longevity has reported the identification of significant differences in tumour-infiltrating immune cells between older and younger individuals (Van Herck et al. 2021). Additionally, Wu et al. (2019) have shown that cancer cells in older individuals have a more aggressive character, partly due to the surrounding environment experiencing a greater level of immune suppression compared to that in younger patients. The authors also confirmed that older patients with cancer exhibited a substantial decrease in adaptive immunity (B cells, T cells, CD8+ T cells, T helper cells, and Treg cells). However, innate immunity (dendritic cells, macrophages, natural killer cells, neutrophils, eosinophils, and mast cells) was upregulated. Elderly cancer patients, however, may have distinct responses to immunotherapy (Wu et al. 2019). In addition, Jin and Hu (2020) also documented that there was a greater presence of several kinds of immune cells, including CD8+ T and CD4+ T cells,

infiltrating the tumour, which was linked to improved clinical outcomes in younger individuals but not in older individuals. From the above findings, it can be inferred that age is a significant risk factor for the occurrence of cancer and it has a direct influence on the treatment outcome of cancer therapy.

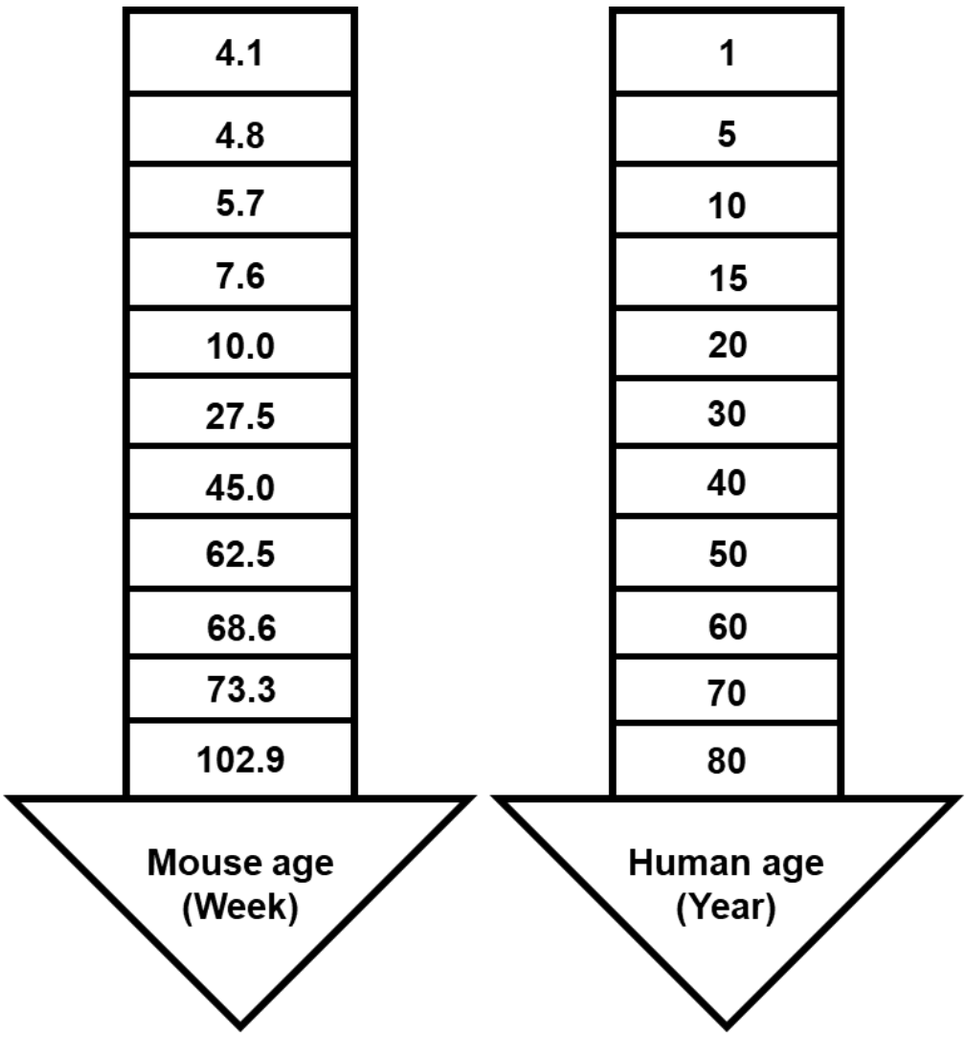

Figure 1. The equivalent age comparison between mouse (in week) and human (in year). The image was created with the information available in Wang et al. (2020).

Preclinical cancer research often overlooks the importance of age. This oversight may hinder the translation of findings to human clinical trials. Mice are often used in preclinical cancer research because their genome has 99% similarity with the human genome, and they exhibit comparable organ structures and systemic physiology (Wang et al. 2020). It is important to note that the mice often used in cancer research are typically 6 to 8 weeks old, which corresponds to the physiological age of around 10 to 15-year-old humans (Figure 1). Using young mice may lead to overly optimistic results in preclinical trials, as they do not accurately represent the physiological state of typical human cancer patients. However, this approach overlooks the fact that different types of tumours manifest at different stages of life, with a specific propensity to arise later in life (Wang et al. 2020). According to Figure 1, the optimal age for initiating cancer-related experiments in mice is around 69 weeks, which is roughly representing a 60-year-old patient. At this stage of development, the mice have reached an age where they can accurately imitate or simulate the physical state of the human body. Juvenile mice may be used in studies focused on young age-related cancers, such as leukaemia. For example, mice which are about 4 to 5 weeks old, which is equal to the age of 1 to < 10 years old in humans, may be used for carrying out studies linked to childhood leukaemia. This is because although leukaemia may develop at any age, it is mostly seen in children between 2 to 6 years old (Cincinnati Children's Hospital 2022). According to Manoharan and Ying (2024), none of the 14 preclinical cancer experiments included in the systematic review

used mice older than 8 weeks. The youngest mouse used was 4 weeks old, while the oldest was 8 weeks old. Among the 14 papers, only one, referred to as a leukaemia research-related study, used NOD-SCID mice between 6 and 8 weeks old (Sharma et al. 2016). This specific age range was considered important in this context. Indeed, this preclinical research on leukaemia was later successfully translated into humans (Brown et al. 2018; Brown et al. 2021).

Researchers should be aware that age significantly influences the treatment response and adverse outcomes of the therapy in both young and elderly mice. An essential factor to consider is that several physiological traits might exhibit increased variability in mice that are older than 25 weeks, as compared to mice that are younger than 25 weeks (Edie 2020). Drug effectiveness investigations that only use young mice may underestimate the toxicity profiles, since it may seem less hazardous in young mice but might have the opposite effect in older mice, which serve as models for elderly cancer patients. This is because the physiological processes in young mice often function optimally, whereas older mice have a higher likelihood of developing age-related disorders such as kidney, liver, heart, brain, and other metabolic abnormalities compared to young mice. Shoji et al. (2016) documented age-related variations in the peripheral and central nervous systems of C57BL/6J mice, which resulted in reduced thermoregulation, neuromuscular strength, and motor function from young adulthood to middle age. However, the gradual decline in motor function can potentially be attributed to the concurrent increase in body weight as the mice age. Ageing-related body weight increase is an important aspect that researchers should consider. The anxiety-like behaviour tends to rise as mice age, although, in older mice, a decrease in anxiety-like behaviour is also seen. The impact of age on behaviour linked to depression is still a subject of debate. Yanai and Endo (2021) observed that physical function in mice gradually decreases, beginning as early as 6 months of age. The cognitive performance begins to decline at a later stage, with significant impairment seen by 22 months of age. Significantly, the process of functional ageing in male C57BL/6J mice begins at earlier stages of life compared to when it occurs in humans. It is indeed a shocking fact that the percentage of unsuccessful conversion of drugs from animal testing to human use has consistently been above 92% for many decades. Some of these failures could be related to either unforeseen toxicity, when safety issues emerge during human clinical trials which were not observed in preclinical studies, or a lack of efficacy (Marshall et al. 2023). The author strongly believes that the age of mice or related preclinical animals could be one of the strong reasons behind the failure of translating preclinical studies into clinical settings. As such, it is very important to design preclinical experiments by including geriatric mice (60 weeks old) groups, especially for toxicology experiments. For efficacy experiments other than juvenile cancers, the author would like to suggest using mice of at least between the age range of 28-45 weeks where this age range represents middle age group patients. This is because according to the latest report by Zhao et al. (2023), cancer incidences in younger individuals are increasing sharply. The incidence of cancer in those under the age of 50 has had a steady global rise of 79%.

Maintaining mice for 60 weeks before the research is expensive, but 6 mice per group are necessary for statistical power. While both efficacy and toxicity-related preclinical studies are equally important, the author suggests focusing on and using geriatric mice more in toxicology studies. This is because many mice efficacy studies focused on developing and treating simple subcutaneous tumours, and in many cases, the mice cannot be kept for more than a month because the tumour burden will exceed 1500mm3, especially in the control group. Besides, most subcutaneous tumour models do not accurately represent the actual location of tumour mass in patients. For example, colon cancer cells are injected under the skin of mice to develop a subcutaneous tumour model. The duration of preclinical efficacy studies typically depends on the research objectives. Many preclinical investigations have reported 21 to 28-day efficacy trials (Xue et al. 2020; Wang et al. 2024), which may not be sufficient because cancer drugs or chemotherapy are usually given to patients in multiple cycles over several months. A subcutaneous tumour-related efficacy study could only be considered a proof-of-concept study. If subcutaneous models are required, the author recommends using at least 12-week-old mice because before this age various steps of the growth process are still ongoing and, in fact, some processes like bone maturation continue after the 12th week until the 26th week (Jackson et al. 2017). If the experimental cancer drug needs to be given for 4 cycles, with each cycle lasting 21 days (a total of 84 days), the subcutaneous model is not the best choice. In this scenario, the author suggests the researchers develop an orthotopic or metastatic tumour model in mice. For example, this can be performed by injecting nasopharyngeal carcinoma metastatic cancer cells into the mouse tail vein to develop a lung metastasis model (Xue et al. 2020) or cell injection into the liver lobe (requires minor surgery to expose the liver) to develop an orthotopic model for hepatocellular carcinoma (Sugase et al. 2020). In this case, it is worth using mice in the age range of 28 to 45 weeks or older.

In conclusion, considering the age of mice in preclinical cancer research is crucial for improving the translational success of preclinical studies. By adopting age-appropriate models, researchers can better replicate human disease conditions, ultimately enhancing the efficacy of clinical trials and patient outcomes. Addressing the age discrepancy in cancer models could also benefit research in age-related diseases such as Alzheimer's and cardiovascular disorders, where similar age-related physiological changes play crucial roles. Therefore, rather than just concentrating on mice aged between 4 and 8 weeks, the research should be expanded to include older mice aged 60 weeks since these older mice more accurately mimic the actual cancer patients.

CONCLUSION

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