Longitudinal evaluation of five nasopharyngeal carcinoma animal models on the microPET/MR platform

Jingjing Shi1 · Zhichao Xue2,3 · Kel Vin Tan1 · Hui Yuan1,4 · Anna Chi Man Tsang2,5 · Sai Wah Tsao2 · Pek-Lan Khong1,6

Received: 23 September 2021 / Accepted: 20 November 2021 / Published online: 4 December 2021
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Abstract

Purpose We longitudinally evaluated the tumour growth and metabolic activity of three nasopharyngeal carcinoma (NPC) cell line models (C666-1, C17 and NPC43) and two xenograft models (Xeno76 and Xeno23) using a micropositron emission tomography and magnetic resonance (microPET/MR). With a better understanding of the interplay between tumour growth and metabolic characteristics of these NPC models, we aim to provide insights for the selection of appropriate NPC cell line/xenograft models to assist novel drug discovery and evaluation.

Methods Mice were imaged by [18F]deoxyglucose ([18F]FDG) microPET/MR twice a week for consecutive 3–7 weeks. [18F]FDG uptake was quantified by standardized uptake value (SUV) and presented as SUVmean tumour-to-liver ratio (SUVRmean). Longitudinal tumour growth patterns and metabolic patterns were recorded. SUVRmean and histological characteristics were compared across the five NPC models. Cisplatin was administrated to one selected optimal tumour model, C17, to evaluate our imaging platform.

Results We found variable tumour growth and metabolic patterns across different NPC tumour types. C17 has an optimal growth rate and higher tumour metabolic activity compared with C666-1. C666-1 has a fast growth rate but is low in SUVRmean at endpoint due to necrosis as confirmed by H&E. NPC43 and Xeno76 have relatively slow growth rates and are low in SUVRmean, due to severe necrosis. Xeno23 has the slowest growth rate, and a relative high SUVRmean. Cisplatin showed the expected therapeutic effect in the C17 model in marked reduction of tumour size and metabolism.

Conclusion Our study establishes an imaging platform that characterizes the growth and metabolic patterns of different NPC models, and the platform is well able to demonstrate drug treatment outcome supporting its use in novel drug discovery and evaluation for NPC.

Keywords Nasopharyngeal carcinoma · Patient-derived xenografts · microPET/MR · Tumour growth pattern · [18F]FDG · Necrosis

Jingjing Shi and Zhichao Xue contributed to the work equally and should be regarded as co-first authors.

This article is part of the Topical Collection on Preclinical Imaging

Pek-Lan Khong
dnrkpl@nus.edu.sg

1 Department of Diagnostic Radiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China
2 School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China
3 Center for Advanced Measurement Science, National Institute of Metrology, Beijing, China
4 Department of Nuclear Medicine, Guangdong Provincial People’s Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China
5 Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong SAR, China
6 Clinical Imaging Research Centre, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
Introduction

Nasopharyngeal carcinoma (NPC) is one of the most prevalent malignant diseases among the population in East and Southeast Asia, particularly in the ethnic Cantonese population in southern China [1]. In 2020, more than 133,000 new cases of NPC were diagnosed leading to approximately 80,000 deaths in the same year [2]. While the prognosis of NPC has largely improved due to advancements in treatment in the past decades [3], more than 10% of patients still develop local recurrence and distant metastasis after primary treatment [4]. Hence, management of advanced and recurrent disease, including the use of novel and targeted therapy, remains an important topic in NPC [5, 6]. Positron emission tomography (PET) imaging has been widely used in the clinics to evaluate metabolic activity in tumours, and it has been found useful in the evaluation of treatment outcome and in prognostication of NPC [7–9]. With the advent of positron emission tomography and magnetic resonance (PET/MR), it is now recommended that NPC patients undergo a one-stop evaluation for both metabolic and anatomical information using PET/MR [10–12]. In order to enable rapid translation of novel cancer drugs from discovery to clinical trial evaluation, the potential of microPET/MR in small animal imaging is of interest for early in vivo testing of these drugs and for performing co-clinical trials [13–15].

To better understand the underlying pathogenesis of the disease as well as to develop novel treatment strategies, patient-derived xenografts (PDXs) have been used as important models in preclinical studies. In NPC research, XenO2117 and XenO17 represent the most widely used PDXs [16] and the other two available PDXs are XenO23 and XenO666 [17]. These four xenografts have been passed for over 25 years and are expected to have already lost their original genetic and pathological properties. Among the conventional NPC cell lines that have been available in the past decade, only C666-1 is Epstein-Barr virus (EBV)–positive [18] and hence, it is able to be more representative of clinical NPC. In the recent years, Tsao’s group has found success in establishing several new NPC cell lines and xenografts for investigation: cell line C17 and NPC43 are two EBV-positive cell lines, which were derived from xenografts of metastatic [19] and recurrent NPC specimens [20], respectively; XenO76 and XenO23 were newly established xenografts derived from primary NPC (7) and recurrent NPC specimens, respectively. Although the genetic features of these new NPC PDXs have been reported [20], the longitudinal growth pattern and the metabolic characteristics still remain unknown. Considering that xenografts are able to retain histopathological and molecular features from the parental tumours [21] and that tumour metabolic heterogeneity has been reported across NPC tumours [22, 23], it is reasonable to assume that PDXs from different patients may have diverse metabolic patterns.

In this study, we characterize and compare the tumour growth and metabolic activity in a time-dependent manner, of three EBV-positive cell line models (C666-1, C17 and NPC43) and two available novel EBV-positive xenograft models (Xeno76 and XenO23) using a microPET/MR system. Based on our findings, we subsequently select the most appropriate model, and through the use of Cisplatin, a well-established drug, we assess the utility of the imaging platform in the longitudinal evaluation of drug response in the tumour model. Our overarching goal is to establish an imaging platform that describes the growth and metabolic characteristics of NPC models, establish tumour imaging metrics and provide insights for the selection of appropriate NPC cell line/xenograft models to assist novel drug discovery and evaluation.

Materials and methods

Cancer cell line models and xenografts

C666-1 cell line was obtained from Professor Dolly Huang (Chinese University of Hong Kong). C17 and NPC43 cell lines were newly established in Tsao’s lab [19, 20]. 10^7 cells were resuspended in 200 μl Matrigel in 1:1 (vol:vol) ratio, and the mixture was subcutaneously injected into the right loin of each NOD.CB17-Prkdcscid/J mouse (male, 4–5 weeks old, n = 5 for each model). For xenograft derived from tumour blocks, tumour from donor mouse was minced into fragments of 2 mm in diameter. Then, wet fragments were subcutaneously implanted in the right loin of recipient mice (male, 4–5 weeks old, n = 5 for each model). All animal experiments were conducted according to the animal license issued by the Hong Kong Department of Health and with the approval of the Committee on the Use of Live Animals in Teaching and Research (CULATR) of The University of Hong Kong.

Drug treatment

Cisplatin (Selleckchem, S1166) was diluted with dimethylformamide (DMF) to a stock concentration of 20 mM and stored in 4 °C avoiding light. Tumour mice were randomized into drug treatment group or vehicle group when tumours reach 50–100 mm^3. Then, the stock solution was further diluted with saline into a concentration of 1 mg/ml and 4 mg/kg Cisplatin was given to animals weekly for consecutive 3 weeks by intraperitoneal injection. Tumour volume was measured three times a week using calipers throughout
the entire duration of treatment. Animals were sacrificed after the treatment period and tumours were harvested for further histopathological study and immunohistochemistry.

**[18F]FDG microPET/MR scan**

After cancer cell injection or tumour fragment implantation, microPET/MR monitoring commenced when the tumour was palpable on each mouse. Mice were anaesthetized using a mixture of medical air and isoflurane (induction 5% v/v; maintenance 2.0 – 2.5% v/v) and placed on the preheated nanoScan® 3 T PET/MR scanner (Mediso Medical Imaging Systems Ltd., Budapest). 9.25 ± 0.37 MBq [18F]deoxyglucose ([18F]FDG) was injected via lateral tail vein. Animals were scanned twice a week for consecutive 3–7 weeks till the humane endpoint using the same protocol (Fig. 1). T1- and T2-weighted imaging (T1WI and T2WI) were performed on all the mice for tumour size assessment (Table S1). A 20-min static PET scan was performed 60 min after injection of radiotracer (Table S2). For drug treatment assessment, [18F]FDG PET scan was performed pre- and post-treatment for tumour uptake comparison.

**Autoradiograph**

Tumour was exposed from the mouse body and marked using tissue dye to match the orientation of PET/MR images. Parts of the tumour were quickly embedded in Tissue-Tek medium (Sakura Co., Ltd., Tokyo, Japan) and snap-freeze in the liquid nitrogen. Frozen tumours were sliced into 10-µm sections using cryostat (CM1950 Leica Biosystems, Germany) and covered with plastic wrap. Tumour slices were placed in a cassette together with a phosphor screen and 1-h exposure was performed to detect the distribution of [18F]FDG. Then, the phosphor screen was scanned using Typhoon5 Biomolecular Imager (GE Amersham, UK) with a resolution of 25 µm. ImageJ was used to further process the images.

**Histological studies**

Each tumour section was cut into 5-µm-thick slices for hematoxylin and eosin (H&E) and immunohistochemical staining. Ki-67 (1:100; Santa Cruz, sc-23, 900) was stained as a cell proliferation marker. To prepare for immunohistochemistry, the antigen was retrieved by immersing tumour slices into the boiling sodium citrate buffer (10 mM, pH 6.0) for 20 min. Tumour slices were incubated with 3% bovine serum albumin at room temperature for 10 min and incubated with primary antibody in a wet box overnight. The slices were incubated with 3% H2O2 for 8 min and secondary antibody was applied to the slices for 1 h. DAB (Dako, Cat. #: K346711-2) substrate was applied to the slices till brown color developed. The slices were dehydrated and mounted with Permount (Dako, Cat. #: S3023). All the slices were scanned with color imaging microscope (Olympus DP74). ImageJ was used to calculate the identified non-necrotic tumour region to the intact tumour region with a fixed threshold and QuPath software (University of Edinburgh, UK) [24] was used to detect the positive cells.

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**Fig. 1** Imaging protocol for different NPC models. Weekly PET/MR imaging started from week 2 or week 10 and ended when mice reached humane endpoint. Tumour metabolism was monitored during the observation period. Animals were sacrificed after imaging and autoradiograph was performed on tumours. Tumours were fixed and sliced for H&E and IHC staining.
Image analysis

Static PET images were reconstructed on the Mediso Medical Imaging Systems using the build-in reconstruction algorithm Tera-Tomo 3D. PET images were anatomically coregistered with MRI images using rigid transformation and fused images were viewed on Interview Fusion version 3.03.089.0000 (Mediso Medical Imaging Systems Ltd.). Volume of interests (VOIs) of liver and tumour were manually drawn on the images. $^{[18F]}$FDG uptake was quantified by standardized uptake value (SUV) as described in the previous publication [25]. For comparable analysis, the hepatic $^{[18F]}$FDG uptake was used as an internal reference background for VOI quantification [26]. The tumour SUVmax and SUVmean were normalized by SUVmean_liver and presented as SUVmax_ratio (SUVmax) and SUVmean_ratio (SUVRmean) using the equation: $SUVR = \frac{SUV_{tumour}}{SUV_{mean, liver}}$ [27].

Statistical analysis

Statistical analysis and figures were performed and drawn using Prism (GraphPad, Inc.). Tumour growth rate was calculated by fitting tumour volume at each time point into the exponential growth equation $Y(t) = Y_0 \times \exp(k \times t)$ [28], where $Y(t)$ is the tumour volume at time $t$, $Y_0$ is the tumour volume at initial timepoint and $k$ is the tumour growth rate. Tumour doubling time was calculated using the exponential growth equation. Data are presented as mean values ± standard errors of the means. Relations between SUV and tumour growth rate, as well as histological staining and SUV, were analyzed using Pearson’s correlation. Comparison between pretreatment and post-treatment was done by two-tailed Student’s $t$-test. A $p$-value lower than 0.05 was considered statistically significant.

Results

Growth patterns of NPC animal models

We found that tumours from cell line models C17, C666-1 and NPC43 started to grow as early as week 2 post-implantation, while tumours from fragment implantation, Xeno76 and Xeno23, needed a relative longer time to initiate growth and to reach humane endpoint. To compare among models, a 4-week observation period was selected for each model when tumours reached a comparable size ($\sim 50–200\text{mm}^3$): week 2 to week 5 for C17/C666-1/NPC43/Xeno76; and week 11 to week 14 for slow growing model Xeno23. Within the observation period, all five models attained varying tumour volumes (Fig. 2a and b). Notably, tumours from NPC cell lines (C17 and C666-1) generally were found to have a faster growth rate and shorter doubling time compared with tumours grown from tumour fragment implantation (Xeno76 and Xeno23). C666-1 had the fastest growth rate of $12.7\pm0.4\%$/day and tumour doubling time of $5.5\pm0.2$ days (Table 1), resulting in tumour oversize at a relative early time point. Xeno76 had a growth rate of $7.3\pm0.8\%$/day and doubling time of $10.1\pm1.5$ days. Xeno23 was the slowest in tumour growth, with a rate of $2.9\pm0.6\%$/day and tumour doubling time of $27.7\pm4.8$ days. After 3–4 weeks, the individual difference of tumour volume were relatively large within C17 and C666-1. However, this did not lead to a big standard deviation in tumour growth rate when fitting into the exponential growth curve, suggesting that the growth pattern is relative stable and distinct for each model.

Repeatability of $^{[18F]}$FDG microPET/MR imaging system for SUV measurement

To investigate the metabolic activity of NPC animal models, we first tested the repeatability of the microPET/MR system. The glucose uptake of liver in mice was reported stable upon fasting condition in several studies [29, 30] and SUVmean_liver has been used as the background to normalize radiotracer accumulation [31]. Thus, in our study, SUVmean_liver was used to examine the repeatability of the microPET/MR imaging system. We analyzed the initial liver uptake and endpoint liver uptake for all five mice models and a total of 50 data points were included in the analysis. Bland–Altman plot (Fig. 3) showed mean difference between the two timepoints was $0.0196$ (95% limit of agreement: $-0.099–0.14$). Coefficient of variation (CoV) for SUVmean_liver between the initial and endpoint was $6.95\%\pm5.02\%$. All the data points were within the agreement interval. No significant differences were observed between the initial and endpoint liver uptake. Results show an excellent consistency of liver SUVmean, suggesting the microPET/MR imaging system is stable and able to produce repeatable results.

Metabolic patterns of NPC animal models

We monitored the longitudinal changes in tumour uptake and found highly variable tumour metabolic patterns across tumour types. SUVRmean and SUVRmax of C17 showed a continuous increasing pattern, while C666-1 showed a decreasing pattern (Fig. 4a) attributed to extensive necrosis confirmed by histology at the endpoint. For Xeno76, NPC43 and Xeno23, there were no obvious overall changes in tumour metabolism over time. To further investigate whether tumour metabolism may change in a later timepoint, we continued to monitor tumour uptake of Xeno76 till week 8 when the mice reached humane
endpoint; still, we did not observe any significant changes in the tumour uptake. After 4-week observation, C17 has the highest SUVRmean (3.19 ± 0.92) and Xeno76 is the lowest (1.57 ± 0.28). There was a significant difference between C17 and Xeno76 in SUVRmean (p = 0.0476), suggesting the metabolic heterogeneity across NPC animal models. We also compared the change in SUVRmean over the 4-week period (Fig. 4b) and found good correlation between growth rate and absolute change in SUVRmean (Fig. 4c, r = 0.6340, p = 0.0009), regardless of tumour types and metabolic patterns. As expected, there was a larger standard deviation in SUVmax than SUVRmean values due to the inherent characteristic of SUVmax compared to SUVmean measurements (Fig. 4b and d).

**Relation between PET, H&E and Ki-67**

To compare PET images and autoradiography with H&E staining, tumour was extracted at the endpoint and tumour slices went through histological analysis (Fig. 5a, b and c). The low uptake region shown on the PET images and autoradiography image was confirmed to be necrotic region by H&E staining (Fig. 5d). We then performed a correlation between tumour SUV and the percentage of the non-necrotic region to whole tumour region represented by H&E staining, across all tumour types. Strong positive correlation was
found between SUVRmean and the percentage of the non-necrotic region (Fig. 5e). Notably, this result revealed that the presence of necrotic regions is a cause of reduced overall \([^{18}\text{F}]\)FDG uptake in the tumour.

In addition, we analyzed the expression level of cell proliferation marker Ki-67 at the endpoint. Proliferating cells mainly accumulated in the outer layer of the tumours, which was consistent with the distribution of higher glycolytic regions on the PET images (Fig. 5a and c). Diverse expression levels of Ki-67 were found among models. The expression level of Ki-67 was the highest in C17 and the lowest in C666-1 (Fig. 5f).

In summary, we found that C17 has an optimal growth rate and high SUVRmean, and this result was consistent with histology, which is high Ki-67 level and little necrosis confirmed by H&E. C666-1 has a fast growth rate, relatively low Ki-67 level, low SUVRmean and much necrosis confirmed by H&E. NPC43 and Xeno76 have slow growth rates and also low Ki-67 level and SUVRmean, as well as extensive necrosis confirmed by H&E. Xeno23 has the slowest growth rate among these models, but it has a relative high Ki-67 level and high SUVRmean. Notably, C17 has a much higher endpoint SUVRmean compared to the other models which had similar SUVRmean (Fig. 5g).

**Treatment assessment**

As a proof of concept, we applied standard chemotherapy for NPC and cisplatin and evaluated tumour growth rate, metabolism and cell proliferation marker Ki-67 before and after the treatment. We selected C17 as the optimal tumour model for our purpose due to the satisfactory growth rate and high tumour metabolic activity which we observed over the 4-week period. 4 mg/kg cisplatin was administered for consecutive 4 weeks. The result of cisplatin treatment is shown in Fig. 6. During the treatment period, we observed a significant tumour inhibition effect by cisplatin. Statistical analysis showed significant differences in tumour volume (Fig. 6a and b) and SUVRmean (Fig. 6c) between treatment group and vehicle group. Immunohistochemistry staining also confirmed the results. Our results showed a remarkably lower expression level of Ki-67 in cisplatin-treated group, suggesting that tumour cell proliferation have been suppressed by cisplatin (Fig. 6d, e and f). Imaging using microPET/MR was able to demonstrate the metabolic and anatomical outcome of cisplatin treatment on the NPC model.

**Discussion**

A successful preclinical study would largely depend on the selection and use of suitable animal models to predict clinical efficacy [32, 33]. As new EBV-positive NPC xenograft models have been established recently [19, 20], more choices are available in selecting tumour models for experiments. The clear characterization of these tumour models would promote the appropriate model selection and therefore facilitate an improved outcome for preclinical studies. We aimed to depict and compare the growth pattern, the metabolic activity and the histological characteristics of these models. Firstly, we found that xenografts derived from cancer cell lines (C666-1 and C17) tend to initiate tumour growth in an early timepoint and have a faster growth rate compared with xenografts derived from tumour blocks (Xeno76 and...
Xeno23) (Fig. 2). This finding is consistent with previous studies done by Tsao’s group [34]. Similar trends were also reported in colorectal cancer [35] and breast cancer [36], and the reason could be as follows: cancer cells which were injected into the mouse could interact with the local environment in three-dimensions and have easy access to the nutrients and oxygen, thus resulting in fast tumour growth [37]. On the other hand, for the implanted tumour blocks, only cells in the outer layer have direct access to the nutrients and oxygen. Furthermore, we also observed different tumour growth rates within the three cell line xenografts: the two well-established models C666-1 and C17 have a similar growth rate that is much faster than newly established NPC43 (Table 1). One potential reason is that both C666-1 and C17 were established from xenografts that have been passaged for over 25 years [16, 18]. During years’ of passaging, highly aggressive cells may be retained and passaged from parental tumours to the next generation, thus causing C666-1 and C17 to exhibit more aggressive growth compared with NPC43. Nevertheless, both C666-1 and C17 are EBV-positive in vitro and representative NPC models for investigation.

Preclinical imaging holds a key role in the non-invasive longitudinal evaluation of therapy response and in the establishment of novel drugs. Traditional drug studies in animal models use “tumour size” as the standard to evaluate drug efficacy. Tumour size when applied as a single indicator has limitations as in most situations it is a surrogate marker for late therapy response compared to change of tumour metabolic activity. For instance, we found that C666-1 and C17 have relatively similar robust growth rates which could be measured through traditional method. However, using the microPET/MR, we first noted that with the similar growth rate, the two models

![Fig. 4 Longitudinal metabolic patterns of NPC animal models using microPET/MR.](image)
actually had diverse metabolic activities (Fig. 4a, b and d). The decrease in tumour uptake in C666-1 can be explained by the early and extensive tumour necrosis which was observed by H&E staining. In contrast, the uptake of C17 was in tandem with increase in tumour size and only relative small necrotic regions were observed at the endpoint. The distinct metabolic patterns may be related to the origin of the tumours: C666-1 was from a primary NPC [18] and C17 was from a cutaneous metastasis of a poorly differentiated NPC [19]. Previous studies suggested that cancer cells experience profound metabolic reprogramming due to the pressures exerted on cells from the microenvironment during metastasis, thus leading to metabolic change and even genomic change from the primary tumour [38]. We found that SUVRmean was highest in the C17 model indicating a higher tumour metabolism, and low in the Xeno76 model, indicating a lower tumour metabolism. These findings were confirmed by cell proliferation marker Ki-67 in the specimen at sacrifice (Fig. 5f), indicating the major advantage of PET in imaging metabolism of NPC animal models. In general, tumour metabolic activity plus tumour size would more comprehensively reflect the status of xenografts.

Animal models are essential for the development of novel targeted therapy in NPC, which has been slow in development partially due to the lack of models, and suitable models that are able to retain their EBV episomes during passaging [20]. A robust animal model for longitudinal studies should not only have a suitable growth rate, but also maintain an appropriate metabolic activity, i.e., without overt necrosis. Based on our findings, C17 and C666-1 both have an optimal tumour growth rate. The advantage of C17 over C666-1 is that C17 can maintain sufficient metabolic activity for the purpose of longitudinal evaluation (Fig. 5g). For C666-1 however, the treatment effect might be compromised by the high level of necrosis. In 2019, Xue et al. [34] showed that
when giving the same dose of palbociclib treatment, C17 presented better treatment outcome than C666-1. A prior study performed by Gressette et al. in 2014 using XenoC17 found that the tumour model was sensitive to the treatment of cisplatin. To evaluate our imaging platform, cisplatin was administrated to C17 cell line xenograft and we found the platform useful to demonstrate the expected therapeutic results of cisplatin. Hence, we suggest C17 could be a robust model for treatment studies. As for C666-1, due to its defective lytic EBV reactivation [39], it can represent a unique group of NPC patients and be used to develop treatment strategies targeting the induction of lytic reactivation. Furthermore, because both cell lines and xenografts are available for C666-1, C17 and NPC43, they can be used to conduct matched in vitro/in vivo studies to test the efficacy of novel drug treatment or targeted therapy [40]. For Xeno76, Xeno23 and NPC43, all these models have a relative slower growth rate and stable tumour metabolic activity throughout the study.

**Fig. 6** Cisplatin treatment on NPC animal model C17. 

- **a** Plot shows the in vivo growth inhibition of C17 tumour by cisplatin. A significant decrease in tumour volume was observed after weekly treatment of cisplatin for continuously 4 weeks ($n = 4$ mice per group). 
- **b** Representative $[^{18}F]FDG$ coronal PET/MR images of mouse bearing with C17 (tumours indicated by white arrows) show the in vivo change in tumour volume and tumour metabolism after cisplatin treatment. 
- **c** Bar chart shows the change in SUVRmean before and after treatment. Statistical analysis reports a significant decrease in SUVRmean after treatment compared with vehicle group ($p = 0.0016$). A significant increase was observed in SUVRmean in the vehicle group ($p = 0.004$). 
- **d** Bar chart shows the change in cell proliferation marker Ki-67 before and after treatment. Cisplatin-treated group shows a significant decrease in Ki-67 positive cells. 
- **e–f** Representative images of H&E and Ki-67 staining of the tumour tissue from treated group and vehicle group. A decrease of proliferating cells was observed in cisplatin-treated group compared with vehicle group ($p < 0.01**$, $p < 0.0001****$).
growth. When comparing Xeno76 and Xeno23, though both models take a long time to initiate tumour growth, Xeno76 grows quickly once the tumour reaches a palpable size and results in large necrotic regions. On the other hand, Xeno23 remains small for at least 3 months with relative less necrotic regions. For NPC43, though it has steady growth rate and metabolic activity, it was found to easily form necrotic regions and extensive cystic changes were noted in several samples (Fig. S1). In case of need for slower growing tumour models, NPC43 and Xeno23 may be considered.

This study has several limitations: firstly, these NPC animal models were not established on humanized immune system mice; thus, the interaction of metabolic activity with the immune system cannot be studied, and hence these models cannot be used to evaluate response to immunotherapies. Apart from traditional chemotherapy, potential targeted therapy for NPC may be tested on this platform, for example, CDK4/6 inhibitors [34] and PI3K/Akt pathway inhibitors [41]. Furthermore, PET imaging is inherently limited in spatial resolution which makes the evaluation of tumour necrosis and heterogeneity challenging.

This study demonstrated the distinct growth and metabolic patterns across different NPC xenografts, as well as illustrating the application of tumour imaging metrics using a microPET/MR system. Further investigations are needed to explore the molecular mechanism that resulted in the distinct characteristics of these tumour models. Moreover, the metabolic characteristics of the specific PDX model have the potential to guide its donor patient’s treatment [42], and thus lead to a new era of personalized medicine.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00259-021-05633-4.

Author contribution JS and ZX contributed to the study conception and design. JS conducted the microPET/MR experiments, image analysis and data analysis. ZX conducted the animal model establishment and histological study. The first draft of the manuscript was written by JS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The research was supported by the University of Hong Kong Seed Fund for Basic Research (201910159081) and the Hong Kong Research Grants Council (RGC) Collaborative Research Grant (C7018-14E).

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval All animal experiments were conducted under conditions compliant with the animal license issued by the Hong Kong Department of Health and with the approval of the Committee on the Use of Live Animals in Teaching and Research (CULATR) of the University of Hong Kong (CULATR No. 4898).

Consent to participate Not applicable.

Consent for publication All authors involved in the study provided their consent to the submission of this article for publication.

Conflict of interest The authors declare no competing interests.

Clinical trial registration Not applicable.

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