The purpose of the present study was to assess if small animal PET is useful for serially monitoring the development of a human anaplastic large cell lymphoma (ALCL) murine xenograft and for the early selection of tumour bearing animals. The human ALCL Karpas 299 cell line was subcutaneously injected in 6-week-old NOD/SCID (non-obese diabetic/NCrCrl-Prkdc) mice (10^7 cells/mouse in 150 µl FBS) at the right flank level. Small animal 18F-fluorodeoxyglucose positron emission tomography (18F-FDG PET) was serially performed (intravenous injected dose: 20 MBq in <0.15 ml, uptake time: 60 min, image acquisition: 1 bed position of 15 min): early PET at 2 days after cell inoculation in 4/8 mice and at 4 days in the remaining 4/8, later PET scans were performed in all the animals at 7, 14, 21 and 28 days after inoculation. The images were evaluated visually and the tumour to background ratio (TBR) was used for semi-quantitative analysis. Pathology sections were obtained in all cases. PET detected the presence of the tumour as early as seven days after inoculation in 4/8 mice and at 14 days in 2/8. Of the two remaining mice, one died after the first PET scan (thus preventing any evaluation of detection time) while the other showed a microscopic neoplastic infiltration at tracheal level at autopsy. Mean TBR progressively increased in all positive cases, particularly in the first 3 weeks, reaching a plateau afterwards. PET was positive in 6/8 (75%) animals, detecting the presence of viable tumour cells earlier than macroscopic evaluation, thus may be used for the early identification of tumour bearing animals.

Primary systemic anaplastic large cells lymphoma (ALCL) accounts for about 5% of all non-Hodgkin lymphomas in adults and 20-30% of large-cell lymphomas in children (1). ALCL represents a heterogeneous group of aggressive non-Hodgkin lymphomas characterized by strong expression of CD30 (cell membrane protein of tumor necrosis factor receptor) and frequent t(2;5) chromosome translocation (2). Notwithstanding its responsiveness to chemotherapy, about 30-40% of the patients die after intensive treatment and standard therapy is associated with considerable toxicity, particularly bothersome in the paediatric population. Therefore alternative new drugs need to be developed not only for relapsing patients but also as first line treatment.

18F-fluorodeoxyglucose positron emission tomography (18F-FDG PET) is widely used for the assessment of lymphoma in human patients for staging, assessment of the response to therapy and the early detection of relapse, influencing in many cases the patients clinical management (3-11). Murine models of human cancer are valid tools to study tumour development over time and to test potentially curative new drugs. Xenograft models in particular are extensively used for new drugs testing for their low cost, reproducibility and similarities with human tumours and murine models of lymphoma are useful tools for pre-clinical studies. In recent years different imaging modalities, traditionally used for humans, such as PET, CT, MRI, optical imaging and single photon emission computed tomography (SPECT) have been designed for animal research and employed in oncological preclinical studies (12-21). For new drug testing small animal PET offers many
advantages over anatomical imaging modalities that rely only on size criteria for the evaluation of therapy response. Among the different imaging techniques, small animal PET shows a good spatial resolution (1.0-1.5 mm) (22) and is the only method allowing sequential evaluation of the same animal over time. PET offers the advantage of non-invasively providing functional information of the tumour lesion and is therefore very accurate for the assessment of the metabolic changes consequent to drug administration. Moreover, PET can detect the presence of viable tumour cells at a very early stage and this feature can be particularly helpful for identifying animals with very small, well vascularized tumours without necrosis, an ideal setting for testing new drugs. However, before testing a new molecule, it is crucial to assess the metabolic characteristics of the tumour over time and the earliest time-point when the lesion can be detected. Small animal PET therefore represents a valid tool for assessing the response to treatment (23). Therefore it is crucial to identify a suitable murine model of disease that can be accurately monitored by PET imaging.

Xenograft models present some limitations mainly related to the formation of a chimeric neoplastic tumour, to the need of an immunocompromised host and, in some cases, to the heterotopic site of tumour formation, nevertheless xenograft models have been widely employed, especially for drug testing studies. On the other hand, transgenic models are orthotopic, but are characterized by an entirely murine tumour mass and are very expensive.

The Karpas 299 xenograft murine model, expressing the NPM-ALK (nucleophosmin – anaplastic lymphoma kinase) fusion gene, has been used in different studies investigating the role of novel molecules for lymphoma therapy (24-26). To our knowledge there are no reports evaluating the role of novel molecules for lymphoma therapy (24-26). The Karpas 299 xenograft murine model, expressing the NPM-ALK (nucleophosmin – anaplastic lymphoma kinase) fusion gene, has been used in different studies investigating the role of novel molecules for lymphoma therapy (24-26). To our knowledge there are no reports evaluating the role of novel molecules for lymphoma therapy (24-26).

The aim of the present study was to assess if a xenograft model with a well established human ALCL cell line could be accurately monitored by small animal PET over time and if PET could be used for early assessment of the presence of the tumour and therefore proposed for the selection of mice bearing tumours early in the course of the disease to start the testing of a new drug, promptly.

Materials and Methods

Cell culture. The human anaplastic large cell lymphoma Karpas 299 cell line (established from a 25-year-old ALCL patient) was purchased from DSMZ (Deutschen Sammlung von Mikroorganismen und Zellkulturen, Germany) (1, 27). The cells were maintained in 90% RPMI medium 1640 supplemented with 10% FBS at 1.0-2.0x10^6 cells/ml (maximal density of about 2-3x10^6 were maintained in 90% RPMI medium 1640 supplemented with 10% FBS at 1.0-2.0x10^6 cells/ml (maximal density of about 2-3x10^6). The cell cultures were kept at 37°C with 5% CO2. The Karpas 299 cells growing exponentially were resuspended in PBS and inoculated into the mice subcutaneously (1x10^6 cells/mouse) at the right flank level.

The whole experiment was approved by the Ethical Committee of the University of Bologna.

Tumour imaging by FDG small animal PET. PET scans were carried out under sevofluorane (5%) anaesthesia (VetEquip Complete Anaesthesia System, Pleasanton, CA, USA) and oxygen supplementation (1L/min). Each anaesthetised animal was injected with 20 MBq of 18F-FDG in the tail vein (injected volume <0.15 ml) under sevofluorane (5%) anaesthesia (VetEquip Complete Anaesthesia System, Pleasanton, CA, USA) and oxygen supplementation (1L/min). Each anaesthetised animal was injected with 20 MBq of 18F-FDG in the tail vein (injected volume <0.15 ml) and subsequently allowed to wake up during the uptake time (60 min). The residual dose in the syringe was measured to verify the effective dose injected. At the end of the uptake time, PET image acquisition was performed with a small animal PET tomograph (GE eXplore Vista DR, Ontario, USA) of the anaesthetised mouse placed prone on the scanner bed. The total acquisition time was 15 minutes. Since the axial field of view was 4 cm, a single bed position was sufficient to cover the whole body. Once the scan was completed, the animal was allowed to wake up in a warmed recovery box.

Early PET imaging was obtained at 2 days from cell inoculation in 4/8 animals and at 4 days in the remaining 4/8 animals. Later scans were performed in all the animals at 7, 14, 21 and 28 days from cell injection.

The FDG PET images were reconstructed iteratively (OSEM 2D, ordered subset expectation maximization) and read in three planes (axial, sagittal and coronal). The scan was considered positive if any area of increased non-physiological FDG uptake was observed. The cold areas located at the centre of a tumour mass were interpreted as areas of necrosis. Semi-quantitative analysis was carried out in all cases using the tumour to background ratio (TBR), placing the target region of interest (ROI) in the most active tumour area and the background ROI in the contra-lateral subcutaneous tissue (TBR=mean count in the target ROI/ mean count in the background ROI).

Histology evaluation. Pathological sections were obtained in all cases. The animals were sacrificed 30 days after tumour cell inoculation (6/8 cases). An autopsy was performed before the end of the study in the two animals that died during the experiment (one mouse died after the first PET and one animal died at 27 days from tumour cell injection). Pathology was used to confirm the PET findings. In all the cases the tumour was excised and samples from the heart, lungs and peritoneum were obtained for
histological evaluation. At macroscopic observation one mouse showed liver and kidney enlargement therefore liver and kidney samples were collected. In one case with all negative PET scans, samples were taken also from the liver and intestine.

The formalin fixed tissue samples were embedded in paraffin and 5 μm sections were cut and stained with hematoxylin and eosin.

Results

The early PET evaluation after tumour cell injection (at 2 days or 4 days) was negative in all cases. Subsequently the small animal PET identified the presence of lymphoma in 6/8 (75%) mice. A pathological FDG uptake at the inoculation site was evident at 7 days after implantation in 4/8 (50%) mice and at 14 days in the remaining 2/8 (25%) animals. Of the remaining two animals, one died after the first PET scan and autopsy did not reveal the presence of a tumour, the PET scans carried out in the other mouse were negative at all the time-points.

In all cases the PET detected the presence of viable tumour cells before macroscopic evaluation. Palpable tumours were observed at 14 days in another 3/8 and at 21 days in 3/8 (overall mean 17.5 days), therefore PET detected the tumour mass an average of 8 days (range 7-14 days) in advance.

The serial PET documented a progressive increase in the metabolic activity of the tumour mass in all the PET positive mice (Figure 1). In the last scans, PET showed cold central lesion areas compatible with necrosis, secondary to tumour vascularisation incompetence in 6/6 cases.

The lesions mean TBR presented a greater increase in the first three weeks, especially between 7 and 14 days, while in the last scans the trend tended to plateau, reflecting necrotic changes in the tumour mass (Figure 2).

Quite interestingly, in the two animals in which the PET turned positive only at 14 days, the mean TBR at 14 days was higher (6.2) then the mean TBR observed in the animals that turned positive at 7 days (4.4).

The pathology sections of the tumour samples (Figure 3) showed in all cases the presence of an aggressive tumour, with pleomorphic lymphoma cells presenting a high mitotic and apoptotic rate. In all the animals the lesions presented a big central necrotic area. The tumour often infiltrated adjacent tissues (muscles, abdomen wall, fat, peritoneal vessels) and organs, including kidneys (in one case), liver (in two cases), intestine (one case) and lung (in five cases). The involved lungs presented diffuse lymphoma cell infiltration with sparse foamy macrophages that filled the alveolar spaces without evident fibrosis. The widespread lung involvement prevented any considerations regarding the pulmonary TBR changes over time.

The autopsy carried out in the single animal in which PET was negative at all time-points revealed the presence of a very small lymphoma infiltration embracing the trachea and esophagus with no macroscopically evident mass neither the tracheal or the inoculation site.

Discussion

Much attention has been directed to therapeutic drugs aimed at down-regulating NPM-ALK fusion protein expression or inhibiting the NPM-ALK signalling cascade (28, 29) and recently small interfering (si)RNA have been used to specifically down-regulate NPM-ALK fusion protein expression in the Karpas 299 cell line “in vitro” inducing decreased cell proliferation and increased apoptosis (30). Accordingly, a murine xenograft model using a well established ALCL cell line, carrying the t(2;5) chromosome translocation was chosen, in order to evaluate tumour development over time by small animal PET.

In our sample, PET detected the presence of the tumour in almost all the animals, identifying the earliest lesions at seven days after cell inoculation in the majority of cases, furthermore in all the mice PET identified the tumour earlier than macroscopic evaluation (average seven days in advance). In fact PET was positive in 6/8 mice, while in the remaining two cases there was no lesion that PET could have detected (one mouse died very early, the other did not develop a mass at the inoculation site).

These data are particularly promising demonstrating the possibility of detecting the presence of a very small tumour at an early stage. The present results were quite interesting when compared with those obtained by Jundt et al., studying the same model macroscopically (26). The authors obtained palpable lesions between one and two weeks, reaching an average tumour volume of 32.7 mm³ at 16 days, while in our series a palpable mass was evident between two and three weeks. Differences in tumour cells phase of growth at the time of inoculation may have accounted for differences in the time of formation of a palpable mass. Moreover, patterns of differential tumour growth may exist among different animals as reported by Sato et al. in five SCID mice injected with Karpas 299 intraperitoneally (31).

The tumour lesions presented progressive increased metabolic activity, as expressed by mean TBR variations, especially in the first three weeks. Afterwards, the TBR reached a plateau which reflected the necrotic changes, evident on the last PET scans, in the central areas of the tumour mass. The higher mean TBR value of the animals with first positive PET at 14 days compared with those with first positive PET at seven days, suggested the need to further assess one week PET-negative animals between 7 and 14 days, since it is likely that the tumour lesion could have been detected earlier.

The pathological examination showed a widespread tissue distribution including lungs, liver, kidneys and intestine, as well as adjacent fat and muscles. The widespread distribution of lymphoma cells in the alveolar spaces was the major reason why the background ROI in the contra-lateral subcutaneous tissue was chosen instead of the lungs.
Figure 1. Serial $^{18}$F-FDG small animal PET images of ALCL development after implantation of human Karpas 299 cells. Early PET scan was negative (A) while PET performed at 7 days from tumour cells implantation showed the presence of pathological FDG uptake area at the inoculation site (B). The metabolic activity of the mass progressively increased over time (C, D).
Figure 2. Tumour to background (TBR) variations over time in PET-positive tumour bearing animals showed a greater increase in the first three weeks. The TBR trend tended to plateau at later time-points reflecting the presence of central necrotic areas.

Figure 3. Pathological sections of a NOD/SCID mouse inoculated with the human ALCL Karpas 299 cell line. The tumour mass showed highly pleomorphic lymphoma cells with a high mitotic and apoptotic rate (A, 60x) and a big central necrotic area (B, 4x). The tumour was very aggressive, infiltrating the lungs and the liver (C, 4x D, 40x).
In one case PET was negative at all time-points while the autopsy revealed the presence of tumour cells at the tracheal level without a macroscopically evident tumour mass. Although a localization of lymphoma cells at the head and neck level has been reported after intravenous Karpas 299 cell administration (32), it is seems unlikely this was the case, since the autopsy did not reveal any other site of tumour involvement that could have been expected after intravenous cell injection. One possible explanation for this unusual localization could be a possible mistaken cell injection directly at the mediastinal level.

**Conclusion**

In this ALCL xenograft murine model small animal PET allows the detection of tumour lesions before macroscopic evaluation at a very early stage.

**References**


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