Clinical and experimental molecular and multimodality imaging studies in B cell malignant lymphomas

(Klinikai és kísérletes, molekuláris és multimodalitású képalkotó vizsgálatok B sejtes malignus lymphomában)

Doctoral (Ph.D.) – thesis

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1 Introduction - Focus on Lymphoma diagnostics

Haematological malignancies can occur at various life stages throughout age groups. Non-Hodgkins's lymphoma (NHL) is globally the most common haematological malignancy, accounting for nearly 3% of cancer diagnoses and deaths. The non-Hodgkin lymphomas are a diverse group of malignancies about 80% of which are of B-cell origin in the Western hemisphere. The most common histologic subtype in adults is diffuse large B-cell lymphoma (DLBCL) composing about 30%-40% of NHLs diagnosed worldwide each year. Around half of the morbidities occur in the age group of patients older than 60 years.

Diffuse large B-cell lymphomas are a particularly heterogeneous group of lymphoproliferative neoplasms with different biology, clinical presentations, and response to treatment. For an effective therapeutic strategy, it is important to understand their heterogeneous phenotypes.

Although outcomes for patients with DLBCL improved significantly with the addition of Rituximab to the standard chemotherapy backbone over a decade ago, they have largely remained stable since that time. Approximately 40 % of DLBCL patients will develop relapsed or refractory disease. Most patients in this setting succumb to their disease despite salvage treatments, suggesting that the highest impact area for improvement is first-line therapy. Summarizing DLBCL is potentially curable with standard treatment in approximately 60% of cases. Standard treatment includes Rituximab, cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulfate (Oncovin), and prednisone (R-CHOP). About 25%-30% of patients are resistant to standard chemo-immunotherapy, therefore other therapeutic approach is utilized, namely 20% of patients are treated with salvage therapy including high dose therapy and autologous hematopoietic stem cell transplantation.

Recent advances in molecular characterization techniques have deepened the understanding of the biology of DLBCL and paved the way for personalized therapies aimed at improving outcomes in this disease. Advances on the understanding of the genetic landscape and molecular features of DLBCL have identified high-risk group with poor response to chemo-immunotherapy. There is an unmet clinical need to identify these high-risk patients as early as possible to apply targeted and more intensive therapy on individualized basis, as the majority of refractory or relapsed patients will eventually die from their disease.

1.1 The importance of radiomics derived from FDG-PET/CT in predicting the survival

Taking in consideration that about 25-30% of patients are primarily resistant to the current first-line treatment with Rituximab based chemo-immunotherapy, identifying the high risk group which will do not respond, has very high priority.

In addition to histological approaches one of these modalities could be the use of conventional and textural parameters derived from the baseline FDG-PET/CT (2-deoxy-2-[18F]fluoro-D-glucose-Positron emission tomography/X-ray computed tomography). Methods to individualize treatment choices are being increasingly employed in different clinical trials, yielding favourable correlations with improved response rates. Studies in the field of cancer imaging research have been actively engaged with radiomics in combination with machine learning.

Radiomics means the extraction of a large number of quantitative features from medical images using advanced mathematical analysis. With well determined image biomarkers, there is a possibility to characterize tumorous regions more deeply and get data which are invisible and unrecognizable for the human eye.

However, radiomics has been reported to be sensitive on various factors such as individual biology, acquisition protocols, choice of delineation, binning and resolution as well as calculation methods, which challenges prior studies to repeat. Nevertheless, standardization proposals such as the Imaging Biomarker Standardization Initiative (IBSI) support the endeavours to report findings in a repeatable way.

1.2 Preclinical studies 1: focus on Cerenkov Luminescence Imaging (CLI)

In connection with recent advances in lymphoma histology and in vivo imaging studies, in situ in vitro imaging imaging-based diagnostic tools have also evolved. The application of these emerging methods holds the promise of timely access to advanced differentiation of DLBCL patient clusters. The identified subgroups of patients would then benefit from quick and tailored immune- or intensive chemotherapy.

The clinical management of the disease involves the evaluation of enlarged lymph nodes. Before starting definitive therapies, further excisional biopsy, and detailed histological analysis of at least one accessible, tumorous part of a suspect lymph node is a mandatory step in the course of diagnostics.

By localising involved lymph nodes, subsequent sampling could benefit from the use of the currently obligatory pre-therapy FDG-PET/CT or ^{67}Ga-citrate scan images to establish therapeutic directions. As an example, identifying, removing, and analysing lymph nodes and inhomogeneous tumour masses using the available Cerenkov light emission of Fluorine-18 isotope after the PET/CT exam would lead to more precise molecular biology evaluation, hence leading to choosing a better therapeutic protocol.

In this direction, our group has previously described the Bc.DLFL1 tumour model as a spontaneous high-grade lymphoma isolated from BALB/c mice, which is reproducibly transplantable via intraperitoneal injection into syngeneic recipients. This model shows preferential tissue distribution to mesenteric lymph nodes and spleen during peritoneal spreading, and propagates via the lymphatic vessels. This well-characterized lymphoma is a suitable model for preclinical investigations, including assessment of novel imaging techniques. The model invariably leads to death 21 days after the animals are inoculated, with 90% of animal deaths occurring between 11 and 14 days. Thus, animals are in early stage before 4 days of lymphoma administration, in advanced stage from day 7 and end-stage from day 11 post intraperitoneal inoculation.

The basic aim of this part of our work was to assess staging possibilities by imaging and monitoring dissemination of Bc.DLFL1 lymphoma in vivo with PET and SPECT (Single photon emission computed tomography) and ex vivo with high resolution Cerenkov luminescence imaging (CLI).

CLI as an advanced imaging technique has a considerable potential for clinical translation. It is an optical imaging technique based on the emission of Cerenkov photons. The Cerenkov photons are emitted by charged particles such as electrons and positrons. It develops when the velocity of these particles is greater than the velocity of light in a medium (such as in water and tissues). Every new CLI application is of high medical interest, especially those using clinically authorised and marketed radiopharmaceuticals or isotopes. One such yet not reported isotope in practical CLI is ^{67}Ga. However, underreported, theoretical calculations show that the high-energy gamma photons emitted during the decay of this isotope could lead to secondary electrons resulting in Cerenkov luminescence in water or in water-containing tissues.

1.3 Preclinical studies 2: the investigation of tumor spreading in early and advanced stage

After intraperitoneal inoculation of Bc.DLFL1, the lymphoma cells attach to lymphocyte-rich regions within the omentum and mesentery, and subsequently disseminate towards the mesenteric lymph nodes.

The detection of peritoneal metastases at an early stage could be a key component of effective therapy for diseases spreading abdominal. The main role of tumour cells in FDG uptake had been already pointed out in a study examining peritoneal tumour spread in mice.

We believed that the previously well described mouse model could be an optimal model for preclinical investigations dealing with translational detection possibilities of peritoneal spreading. Therefore, we aimed to investigate the early spreading of Bc.DLFL1. lymphoma and compare its detectability in advanced stage, with in vivo FDG-PET and PET/MRI, and ex vivo by autoradiography and CLI using FDG. The results of these imaging techniques were correlated with immunohistochemical tissue analysis.

2 Objectives

In my PhD work, I have used all those imaging methods, which are helpful in the diagnosis and assessment of response to therapy in patients with lymphoma. The dissertation can be divided into a clinical and a preclinical part. Based on the literature, radiomics derived from PET images may contribute to better characterization of tumour in vivo and therefore could help in individualized tumour management. I begin this paper by outlining a related clinical hypothesis. During clinical management, it may occur that the diagnosis is delayed due to an inadequate tissue sampling. In the first preclinical study, we investigated an image-based sampling method using a new and accurate mouse model (spontaneous lymphoma). The spreading mechanisms and routes of lymphoma cells are still unclear. My aim was also to characterise tumour spread and heterogeneity in our rodent experiments both in the first and second part of the preclinical examinations. New diagnostic possibilities emerging from our clinical and preclinical research will be presented based on our published literature.

1. In the clinical study, we deal with the molecular imaging diagnostics of diffuse large B cell lymphoma. In DLBCL despite well-established therapy protocols, 25-30% of patients are resistant to standard chemo-immunotherapy mostly due to heterogeneous origins of the disease. For the identification of high-risk patients in diffuse large B-cell lymphoma we investigated the prognostic significance of in vivo radiomics derived from baseline FDG-PET/CT and clinical parameters utilizing automated machine learning.
2. In the first preclinical study, first we investigated the novel Cerenkov luminescence imaging, which is a promising approach to image-guided surgery and pathological sampling. It could offer additional advantages when combined to whole-body isotope tomography. We aimed to obtain evidence of its applicability in lymphoma patho-diagnostics, thus we decided to investigate the radiodiagnostic potential of combined PET or SPECT/CLI in an experimental, novel spontaneous high-grade B-cell lymphoma mouse model (Bc.DLFL1).
3. In the second preclinical study we followed the early stage spread of Bc.DLFL1 mouse lymphoma model with different imaging modalities. Thorough our investigations we aimed to confirm the importance of local FDG administration during diagnostic imaging, to precisely assess early peritoneal manifestations.

2.1 The related main targeted points

1. Analysis of a 2-years-event-free prediction model using radiomics derived from pre-treatment FDG-PET/CT of patients with DLBCL

2. Development of a novel imaging tool combining PET/SPECT and CLI to be able to detect the most relevant affected lymph node and/or the most relevant part of the affected lymph node in a mouse model.

3. Select the most optimal nuclear medicine technique for early detection of peritoneal lymphoma spread using a mouse model.

3 Materials and methods:

3.1 Clinical part

The studies involving human participants were reviewed and approved by the appropriate local institutional research ethics committee and the Hungarian National Institute of Pharmacy and Nutrition under permission number 6536 – University of Pécs 2017 and OGYÉI/50268-8/2017. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Pre-treatment [18F]FDG PET/CT scans of 85 patients diagnosed with DLBCL were assessed. The scans were carried out in two clinical centers. Two-year event-free survival (EFS) was defined. Based on this criterion, patients were selected into two groups. In Group 0, the patients had no events during the 2-years follow up, and in group 1 the patients had primary refractory disease or relapsed during the 2-years period. After delineation of lymphoma lesions, conventional PET parameters and in vivo radiomics were extracted. Prominent clinical data were also summarized. For 2-year EFS prognosis assessment, the Center 1 dataset was utilized as the training set and underwent automated machine learning analysis. The dataset of Center 2 was utilized as an independent test set to validate the established predictive model built by the dataset of Center 1.

3.2 Preclinical studies 1

The study was approved by the Local Ethics Committee of the University of Pécs. Furthermore, all procedures involving live animals were carried under permission of the National Food Chain Safety Office of Hungary, Department of Animal Health, under license number BA02/2000-16/2015.

3.2.1 Experimental animal model of Bc.DLFL1 lymphoma propagation

All studies reported here have been carried out in accordance with the rules on animal welfare and regulations on other respective subjects in vigour in Hungary. The study had been designed and performed in compliance with the ARRIVE guidelines. BALB/c mice bred at the SPF Animal Breeding Unit of the University of Pécs, Department of Immunology and Biotechnology, aged between 8-12 weeks, were used as lymphoma recipients. After retrieval, the mice were adapted to conventional animal facility of the Department of Immunology and Biotechnology. The Bc.DLFL1 lymphoma cells were maintained as serial intraperitoneal passage.

3.2.2 Grouping and number of animals by tumour stage

In both the FDG-PET and the ^{67}Ga-citrate SPECT/MRI experiments animals were grouped into early stage (imaging 4 days post inoculation of lymphoma cells), advanced stage (imaging 8 and 9 days post inoculation) and terminal stage (imaging 11 and 12 days post inoculation). (Altogether 36 mice at inoculation were grouped into six groups with six mice per group.) Each stage groups contained n=6 animals per radiopharmaceutical. However, in the end-stage DLBCL group of animals at 11 days post inoculation, deaths occurred thus five animals were amenable to the combined in vivo tomographic - ex vivo CLI imaging from the FDG-PET group, and one animal was usable for the ^{67}Ga-citrate SPECT/MRI imaging from its group of originally six animals.

We monitored the lymphoma dissemination at early stage, and at clinically relevant stages such as advanced stage and terminal stage with in vivo 2-deoxy-2-[18F]fluoro-d-glucose (FDG) positron emission tomography (PET)/magnetic resonance imaging (MRI) and 67Ga-citrate single photon emission computed tomography (SPECT)/MRI. In vivo imaging was combined with ex vivo high resolution CLI. The use of CLI with 18F-Fluorine (F-18) and 67Ga-Gallium isotopes in the selection of infiltrated lymph nodes for tumour staging and pathology was thus tested.

3.3 Preclinical studies 2

The mice were grouped into an early-stage group where imaging was performed one day post inoculation of lymphoma cells (n=18 mice), and into an advanced stage group with imaging of n=3 mice performed 7 days post inoculation of the cells.

In the early-stage group, nine animals received intraperitoneal injection of FDG and equally, nine mice received FDG via the intravenous administration route in the lateral tail vein. The advanced stage group received intravenous FDG injections.

In vivo [18F]FDG-PET and [18F]PET/MRI, and ex vivo by autoradiography and Cherenkov luminescence imaging (CLI) were performed. Fiber-optic confocal endomicroscopy imaging of FITC (Flurescein-5-isothiocyanate) molecules and immunohistochemistry were also done.

4 RESULTS

4.1.1 Patient data

At the end of the standard induction therapy, 55 patients achieved complete metabolic remission. During the 2-year follow-up, 14 patients had primary refractory disease, 14 patients relapsed within 12 months, and 2 patients had relapsed between 12 and 24 months. In summary, after the end of therapy, 30 patients had detectable metabolically active tumor tissue and relapsed within 24 months (Figure 1).

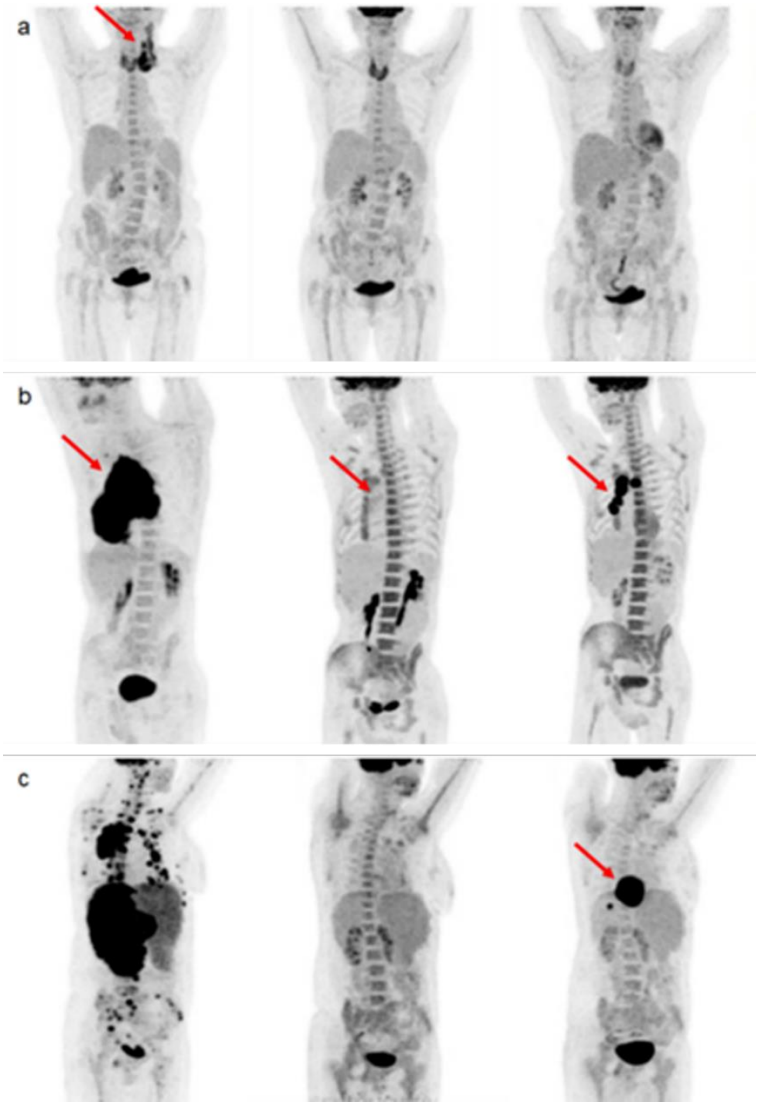

Figure 1: Comparison of clinical outcomes based on maximum intensity projection (MIP) images in three patients (a-c). By each patient, the first image shows primary staging, the second shows interim PET scan, and the third shows post-treatment restaging scan. The red arrows indicate FDG avid lymphoma foci. (a): Patient in complete remission to treatment. The increased FDG uptake in all three images were a sign of thyroiditis. (b): Patient without complete remission during, and after the therapy.

The interim scan showed Deauville score 4. (c): Patient had an interim scan with Deauville score 3 but relapsed after the treatment.

4.1.2 Statistical analysis

Using the data of Chi-square test, a significant association between Cell of Origin (COO), R-IPI (Revised International Prognostic Index), or stages and previously described two specific groups (0 or 1) was identified. There were significantly more patients in group 1 with non-GCB subtype, who had higher R-IPI values and stages. No significant difference was detected between the patients' sex and the specific groups. The clinicopathological features of patients are described in table 1.

Table 1: Comparison of clinical outcome of the patients and their clinical data. Chi square test were performed to find association between the outcome and the specified clinical status of the patients suffering in DLBCL.

Variables	**No Progression or Remission**	**Progression within 24 months**	**p-value**
Sex, n=85	**(n=55)**	**(n=30)**	
Male (n, %)	28 (32.9%)	17 (20%)	**0.611**
Female (n, %)	27 (31.8%)	13 (15.3%)	
ECOG, n=83	**(n=55)**	**(n=28)**	
0 (n, %)	16 (19.3%)	6 (7.2%)	
1 (n, %)	26 (31.3%)	8 (9.6%)	**0.113**
2 (n, %)	11 (13.3%)	12 (14.5%)	
3 (n, %)	2 (2.4%)	2 (2.4%)	
Stage, n=85	**(n=55)**	**(n=30)**	
1 (n, %)	10 (11.8%)	0	
2 (n, %)	17 (20%)	5 (5.9%)	**0.017**
3 (n, %)	9 (10.6%)	8 (9.4%)	
4 (n, %)	19 (22.6%)	17 (20%)	
R-IPI, n=85	**(n=55)**	**(n=30)**	
0 (n, %)	7 (8.2%)	1 (1.2%)	
1 (n, %)	29 (34.1%)	9 (10.6%)	**0.015**
2 (n, %)	19 (22.6%)	20 (23.5%)	
COO, n=82	**(n=53)**	**(n=29)**	
GC (n, %)	27 (32.9%)	7 (8.5%)	**0,018**
N-GC (n, %)	26 (31.7%)	22 (26.8%)	

4.1.3 Automated machine learning analysis and biomarker identification

Automated machine learning yielded 66% sensitivity, 77% specificity, 78% positive predictive value, 70% negative predictive value, 71% accuracy and 0.74 AUC single-centre cross-validation performance in Centre 1.

Feature ranking revealed that the most important features for building 2-years event free survival prediction are: max diameter (9%), NGTDM (Neighbourhood gray-tone difference matrix) busyness (9%), TLG (8%), TMTV (8%) and NGTDM Coarseness (5%). The distributions of these parameters are plotted on violin plots (Figure 2).

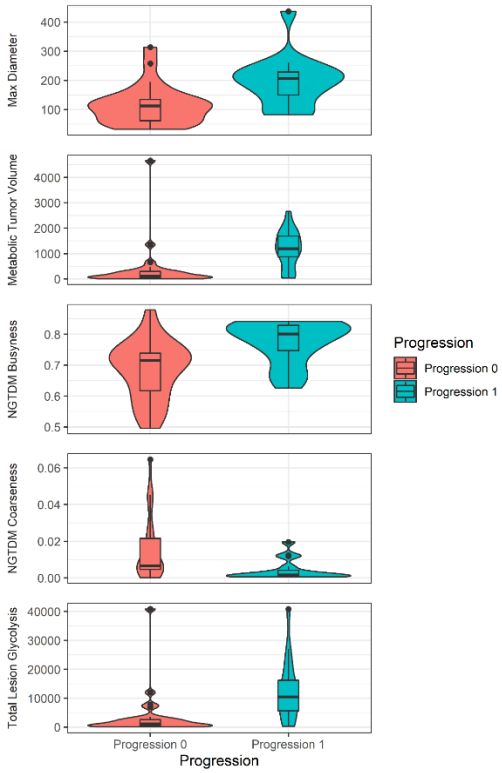

Figure 2: The Violin plot (R: A Language and Environment for Statistical Computing, version 4.04., using package ggplot2, version 3.3.3) shows the values of the prominent features to predict 2-year event-free survival.

4.1.4 Independent validation of prediction model

The predictive model built on Centre 1 dataset yielded 79% sensitivity, 83% specificity, 69% positive predictive value, 89% negative predictive value, 82% accuracy and 0.85 AUC by evaluating the Centre 2 dataset.

4.2 Preclinical studies 1: focus on CLI imaging

4.2.1 Characterization of FDG uptake is feasible both in vivo with PET/MRI and ex vivo with CLI in advanced stage lymphoma

Using in vivo PET/MRI we found that in an advanced lymphoma stage (at or beyond day 8 post-injection) the infiltrated mesenteric lymph nodes clearly showed a significant accumulation of FDG following intravenous administration. With CLI we observed FDG accumulation in the infiltrated adipose tissue along the mesenteric vessels, within the enlarged mesenteric lymph nodes, in the omentum and also in the splenic hilus. Subsequent histological analysis of the FDG signal-positive regions confirmed large infiltrates of lymphoma cells at these sites (Figure 3). Flow cytometric analysis was also confirmed the lymphoma infiltration.

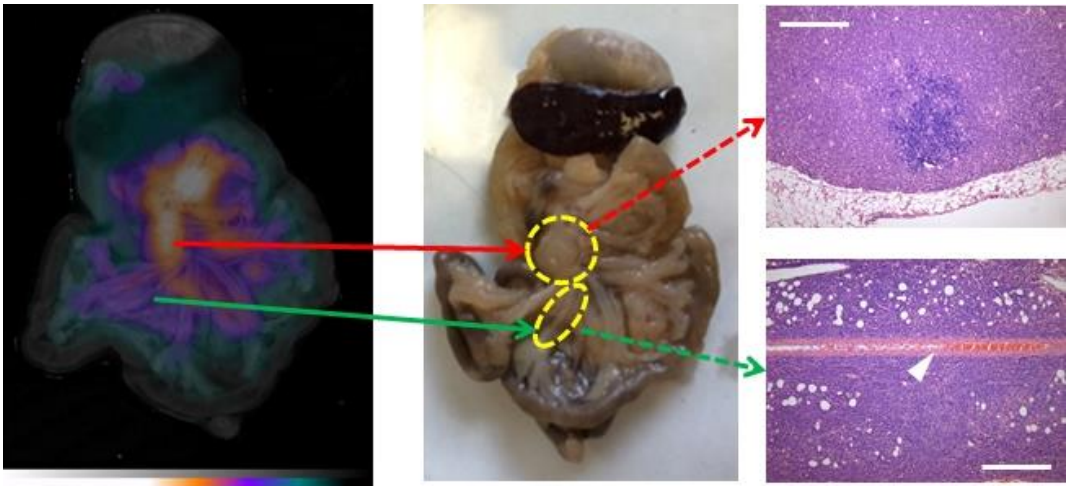

Figure 3: Ex-vivo CLI in late stage lymphoma: FDG-avid Bc-DLFL.1 lymphoma foci with H&E staining in mesenteric lymph node (top) and mesenterium (bottom) CLI (left) and, Stereomicroscopic picture of the intestinal preparation (middle), Histology (right): the red arrow shows that the upper encircled area in the stereomicroscopic picture emits Cerenkov light and corresponds to the mesenteric lymph node complex, containing a massive lymphoma infiltrate demonstrated on the top right image (connected by dashed red arrow). The bottom green arrow connects one branch of the mesentery with lesser Cerenkov signal intensity (marked with ellipse), with an extensive lymphoma infiltrate surrounding the mesenteric artery (arrowhead). Scale bar = 200 μm.

4.2.2 Ex vivo CLI discerns tumorous infiltrate within lymph nodes identified as distant metastases using ^{67}Ga-citrate SPECT/MR

At the advanced stage, the enlarged mesenteric lymph nodes are well detectable using MRI. The distinction of ^{67}Ga-citrate accumulation signals in the lymph nodes based on the SPECT alone without MRI would be difficult due to the high background activity caused by the intense non-specific intestinal accumulation of the tracer. On the other hand, the mediastinal region lacks this background. This allows specific detection of distant manifestations with SPECT, even in tissues without obvious MR alterations. Here the SPECT assessment could clearly reveal lymphoma accumulation in the region of parathymic lymph nodes, which were not well noticeable using only MRI analysis in the end-stage lymphoma-bearing mouse. The presence of lymphoma infiltrate in parathymic lymph nodes was verified following their removal by using ex vivo CLI and subsequent histological analysis, including dual immunofluorescence labelling for IgM and B220.

4.3 Preclinical studies 2: the investigation of tumor spreading in early and advanced stage

4.3.1 Monitoring of early distribution of Bc.DLFL1 lymphoma cells after intraperitoneal inoculation using systemic and topical FDG administration

The typical radiotracer-based approach for in situ tumour detection employs the preferential uptake and cellular entrapment of FDG molecules by tumour cells. The high glucose utilization by most types of tumour cells is well-known.

To determine whether in vivo PET imaging methods would allow monitoring of disease progression at the early stage, we used FDG for high grade lymphoma detection. We injected the Bc.DLFL1 lymphoma cells intraperitoneally, followed by the administration of the radiopharmaceutical 24 hours later either via intraperitoneal or intravenous injection. Using whole body PET scan we could not detect specific tracer accumulation in the early stage (1 day post-inoculation) after either intraperitoneal or intravenous tracer administration. To ensure that the tracer did reach the potential omental and mesenteric propagation sites, next we performed ex vivo autoradiography of the entire intestinal tract. We found that 2 hours after intraperitoneal FDG administration, several clearly marked foci were present within the mesentery or omentum in mice injected with Bc.DLFL1 cells; on the other hand, scanning after the intravenous injection of FDG no such pattern was detectable (Figure 4).

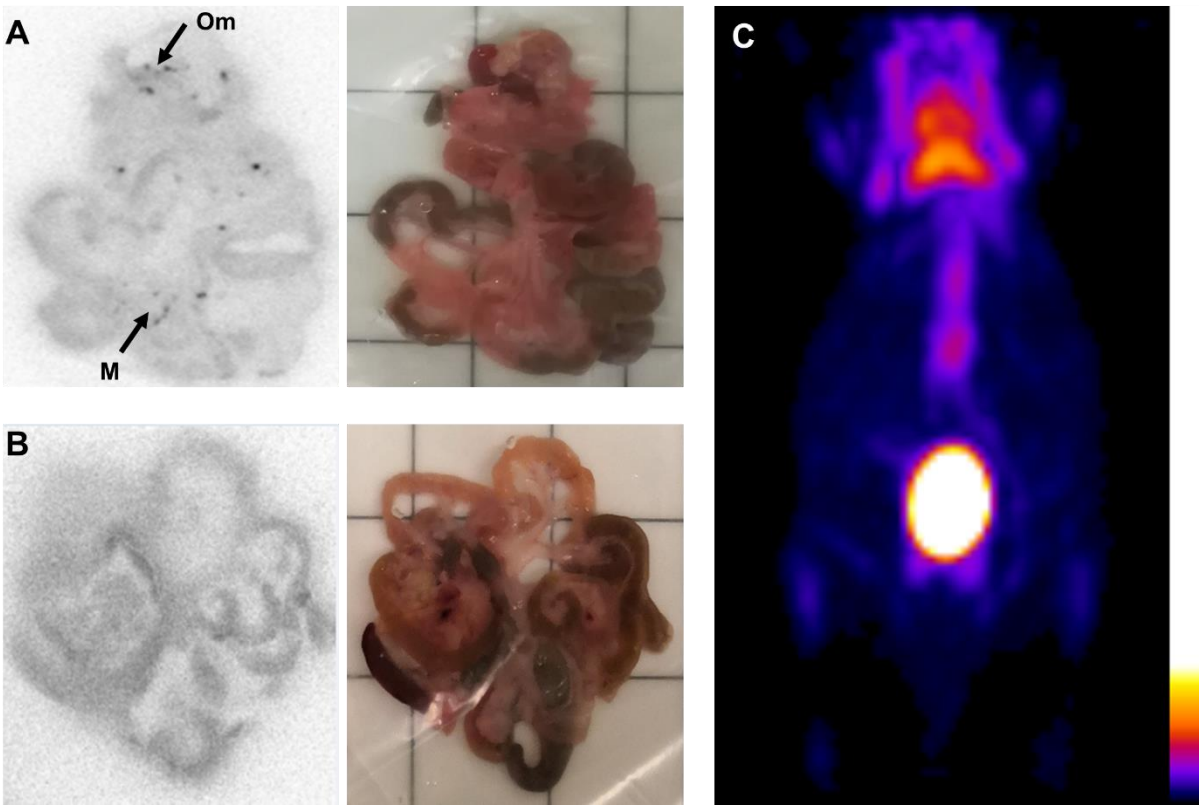

Figure 4: Intraperitoneal FDG is superior in detection to intravenously administered molecules in the early stage Bc.DLFL1 mouse lymphoma model. Ex vivo autoradiographic detection of the tumour cells 24 hours post intraperitoneal inoculation using i.p. FDG injection reveals cells adhered to the omentum (Om) and mesentery (M) placed to the ARG cassette (A). Intravenous administration of the radiotracer fails to identify mesentery- and omentum-associated lymphoma cells (B). The corresponding white light image panel is also presented for orientation (A-B). The lack of tumour-derived signal 24 hours after lymphoma inoculation is shown with a PET three-dimensional maximal intensity projection reconstruction acquired 2 hours following intraperitoneal FDG application. Only normal FDG mouse distribution is visible in the PET image (C).

To confirm that the selective focal omental and mesenteric ex vivo FDG signal is due to the previous local accumulation of lymphoma cells, we intraperitoneally injected BcDLFL.1 cells previously labelled with CFSE (5-6-carboxyfluorescein succinimidyl ester), and their distribution was monitored by immunohistochemical detection with an anti-FITC antibody. Using this technique, we found a labelling pattern matching the distribution observed by autoradiography in the omentum and the perivascular adipose cuffs surrounding the mesenteric arteries, as reported earlier. Thus, our in situ whole mount autoradiography results following intraperitoneal administration of FDG support the immunohistochemical data on the non-random early distribution of lymphoma cells. As a further confirmation of selective in vivo binding of CFSE-labelled lymphoma cells to the serosal lining, we used ex vivo confocal fiber

optic fluorescent microscopy. We found that within the mesentery the CFSE-marked lymphoma cells show focal accumulation in a pattern like that observed with anti-FITC immunohistochemistry. Taken together, these findings indicate that although Bc.DLFL1 lymphoma cells clearly adhere to select peritoneal locations, their FDG tracing after either intravenous or intraperitoneal isotope administration does not permit in vivo lymphoma detection, while ex vivo FDG monitoring can reveal lymphoma accumulation after intraperitoneal tracer administration.

4.3.2 Successful monitoring of lymphoma expansion in nodal metastasis by PET/MR and subsequent CLI in advanced stage

After 7 day of lymphoma cell inoculation. We found that, in contrast to the lack of radiopharmaceutical signal at the early lymphoma stage, a robust accumulation of FDG was detectable following the intravenous administration of tracer. The most intense labelling was observed using PET/MRI in the enlarged mLNs (Mesenteric lymph nodes). To improve the resolution of PET imaging of mesenteric branches, we employed Cerenkov Luminescence imaging as an ex vivo imaging modality with improved resolution. Using this approach we found luminescence signal linked to FGD accumulation in the omentum and in the adipose tissue along the mesenteric vessels and lymphatics, in addition to the intense signal from the enlarged mLNs. These findings reveal that at a later stage of lymphoma, the nodal metastases can be identified using PET/MRI even after the intravenous administration of tracer, but with only marginal separation of lymph nodes and surrounding adipose tissues of the mesenterium. On the other hand, application of CLI as an ex vivo test can sufficiently identify both nodal and adipose lymphoma infiltrates.

5 Summary: the new results of the thesis

1. Analysis of a 2-years-event-free prediction model using radiomics derived from pre-treatment FDG-PET/CT in patients with DLBCL.

Based on our dual-centre retrospective analysis, predicting 2-year EFS built on imaging features is feasible by utilizing high-performance automated machine learning. The most important image biomarkers for survival prediction were max diameter, TMTV, NGTDM coarseness, NGTDM busyness, and TLG. The SUV max, and clinical parameters were less prominent in our analysis. Therefore, subsequent DLBCL studies shall further evaluate the identified imaging biomarkers and their predictive performance in other clinical settings.

2. Development of a novel imaging tool combining PET/SPECT and CLI to be able to detect the most relevant affected lymph node and/or the most relevant part of the affected lymph node in a mouse model.

These results show that in vivo whole-body imaging with PET or SPECT, followed by ex vivo CLI acquisitions might be guiding tumour resection for pathology evaluation and therapy planning not only in preclinical investigations but for the clinical practice, too. CLI could be applied to outline tumorous tissues and to guide their surgical resection. Ex vivo CLI may guide the pathology sampling of any enlarged lymph nodes that have been previously localised by PET. Other surgically removed lymph nodes can be further investigated ex vivo. In this study context, we report Cerenkov imaging of ^{67}Ga isotope for the first time. The combined use of SPECT, PET and CLI in clinical oncological practice is warranted.

3. Select the most optimal nuclear medicine technique for early detection of peritoneal lymphoma spread using mouse model

Our clinically translatable findings point to the early detection possibilities and non-random metastatic route preferences of this lymphoma model. Local FDG administration should be given more consideration in radioisotopic diagnostic procedures. The adequate assessment of early peritoneal metastases in lymphoma, and perhaps clinically more importantly in colon, stomach or ovarian cancers, might be achieved this way. Topical diagnostic applications and the combination of imaging modalities are a clinically explorable method to promote more effective personalized topical therapy, in addition to systemic treatments. Cerenkov imaging translated to the clinic is one such avenue to achieve this.

6 Acknowledgments

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I am grateful to Prof. Dr. Péter Németh for his great suggestions to my publications and research work. In additon to I owe many thanks for the other co-authors of my publications, and I would like to mention here Dr. Ferenc Budán, who helped me a lot with his suggestion to our work.

This work could not have been completed without the supportation of my wife and family.

7 Publications related to the dissertation

1. Zsombor Ritter, László Papp, Katalin Zámbó, Zoltán Tóth, Dániel Dezső, Dániel Sándor Veres, Domokos Máthé, Ferenc Budán, Éva Karádi, Anett Balikó, László Pajor, Árpád Szomor, Erzsébet Schmidt and Hussain Alizadeh, Two-Year Event-Free Survival Prediction in DLBCL Patients Based on In Vivo Radiomics and Clinical Parameters.

Frontiers in Oncology (2022) https://doi.org/10.3389/fonc.2022.820136 Q1, IF: 6,244

2. Zsombor Ritter, Katalin Zámbó, Péter Balogh, Dávid Szöllősi, Xinkai Jia, Ákos Balázs, Gabriella Taba, Dániel Dezső, Ildikó Horváth, Hussain Alizadeh, David Tuch, Kunal Vyas, Nikolett Hegedűs, Tibor Kovács, Krisztián Szigeti, Domokos Máthé & Erzsébet Schmidt, In situ lymphoma imaging in a spontaneous mouse model using the Cerenkov Luminescence of F-18 and Ga-67 isotopes.

Scientific Reports (2021) 11:24002 https://doi.org/10.1038/s41598-021-03505-3 D1, IF: 4,379

3. Zsombor Ritter, Katalin Zámbó, Xinkai Jia, Dávid Szöllosi, Dániel Dezso, Hussain Alizadeh, Ildikó Horváth, Nikolett Hegedus, David Tuch, Kunal Vyas, Péter Balogh, Domokos Máthé and Erzsébet Schmidt, Intraperitoneal Glucose Transport to Micrometastasis: A Multimodal In Vivo Imaging Investigation in a Mouse Lymphoma Model

International Journal of Molecular Sciences (2021) 22(9): 4431

https://doi.org/10.3390/ijms22094431 D1, IF: 5,924

Summarized IF: 16,546

7.1 Other Publications:

1. Zsolt Szakacs, Amar La Jorgen Kristensen, Nelli Farkas, Zsombor Ritter, Szabolcs Kiss, Anett Baliko, Hussain Alizadeh, 90Y-ibritumomab tiuxetan in B-cell non-Hodgkin lymphomas: Real-world data from the United Arab Emirates.

Advances in Radiation Oncology (2021) https://doi.org/10.1016/j.adro.2021.100882 Q2, IF: 2,655

2. Laszlo Szabo, Richard Molnar, Andras Tomesz, Arpad Deutsch, Richard Darago, Timea Varjas, Zsombor Ritter, Jozsef L. Szentpeteri, Kitti Andreidesz, Domokos Mathe, Imre Hegedüs, Attila Sik, Ferenc Budan, and Istvan Kiss, Olive Oil Improves While Trans Fatty Acids Further Aggravate the Hypomethylation of LINE-1 Retrotransposon DNA in an Environmental Carcinogen Model.

Nutrients (2022) 14:908. https://doi.org/10.3390/nu14040908 D1, IF: 5,719

Summarized IF: 8,374

7.2 Published Abstracts:

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2 Ritter Z, Zambo K., Balogh P., Szollosi D., Xinkai J., Dezso D., Alizadeh H., Horvath I., Hegedus N., Mathe D., Schmidt E. The significance of intraperitoneal administration of 18F-FDG in a preclinical mouse model EUROPEAN JOURNAL OF NUCLEAR MEDICINE AND MOLECULAR IMAGING (1619-7070 1619-7089): 47 SUPPL 1 pp S644-S645 (2020)

3 Dezso D., Zambo K., Ritter Z., Ban Z., Szabo Z., Bodis B., Varady E., Szukits S., Toth A., Nemes O., Rucz K., Szujo S., Mezosi E., Bajnok L., Schmidt E., Measurement of epicardial adipose tissue with FDG-PET/CT in patients with type-2 diabetes mellitus EUROPEAN JOURNAL OF NUCLEAR MEDICINE AND MOLECULAR IMAGING (1619-7070 1619-7089): 46 SUPPL 1 pp S465-S465 (2019)

4 Ritter Z., Zambo K., Dezso D., Szabo Z., Ban Z., Kajtar B., Farkas K., Szomor A., Hussain A., Schmidt E., Clinical and prognostic significance of FDG-PET derived biomarkers in high grade B cell lymphoma. EUROPEAN JOURNAL OF NUCLEAR MEDICINE AND MOLECULAR IMAGING (1619-7070 1619-7089): 46 SUPPL 1 pp S565-S565 (2019)

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