differences in the metabolism of the two agents: zoledronic acid retains in the skeleton for years while denosumab does not incorporate in bone [8,12,16].

Recently, a study on 1822 patients with bone metastases from multiple myeloma, breast cancer or prostate cancer compared zoledronic acid administered every three months to the standard monthly dosing. The less frequent dosing was shown to be non-inferior as the risk of SREs was similar during the three-monthly and monthly dosing of zoledronic acid. ONJ developed twice as often during the monthly as the three-monthly dosing [17]. Comprehensive data on the less intensive dosing of denosumab is lacking. REDUCE is a large phase III study currently recruiting prostate and breast cancer patients with bone metastases which randomizes patients to monthly or three-monthly dosing of denosumab.

The risk of medication associated ONJ during bone-targeted therapies seems higher in clinical practice as compared to that reported in randomized trials. Our single institution case series found an 11.4% frequency of ONJ among prostate cancer patients. Only one third of the ONJ cases showed marked improvement whereas the rest caused chronic disability. ONJ is a common and often chronic complication of denosumab and zoledronic acid and more effective preventative measures are warranted.

Disclosure statement

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References

1. Saad F, Gleason D, Murray R, et al. Long-term efficacy of zoledronic acid for the prevention of skeletal complications in patients with metastatic hormone-refractory prostate cancer. J Natl Cancer Inst. 2004;96:879–882.
2. Smith M, Halabi S, Ryan C, et al. Randomized controlled trial of early zoledronic acid in men with castration-sensitive prostate cancer and bone metastases: results of CALGB 90202 (alliance). J Clin Oncol. 2014;32:1143–1150.
3. James N, Sydes N, Mason M, et al. Docetaxel (Doc) +/− zoledronic acid (ZA) for hormone-naive prostate cancer: First overall survival results from STAMPEDE & treatment effects within subgroups (NCT00268476). Abstract 19LBA, European Cancer Congress; 2015.
4. Fizazi K, Carducci M, Smith M, et al. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. Lancet. 2011;377:813–822.
5. Marx R. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg. 2003;61:1115–1117.
6. Ruggiero S, Mehrotha B, Rosenberg T, et al. Osteonecrosis of the jaws associated with the use of bisphosphonates: a review of 63 cases. J Oral Maxillofac Surg. 2004;62:527–534.
7. Zhang X, Hamadeh I, Song S, et al. Osteonecrosis of the Jaw in the United States Food and Drug Administration's Adverse Event Reporting System (FAERS). J Bone Miner Res. 2016;31:336–340.
8. Stopeck A, Fizazi K, Body J, et al. Safety of long-term denosumab therapy: results from the open label extension phase of two phase 3 studies in patients with metastatic breast and prostate cancer. Support Care Cancer. 2016;24:447–455.
9. Ruggiero SL. Diagnosis and staging of medication-related osteonecrosis of the jaw. Oral Maxillofac Surg Clin North Am. 2015;27:479–487.
10. Thumbigere-Math V, Tu L, Huckabay S, et al. A retrospective study evaluating frequency and risk factors of osteonecrosis of the jaw in 576 cancer patients receiving intravenous bisphosphonates. Am J Clin Oncol. 2012;35:386–392.
11. Bamias A, Kastritis E, Bamia C, et al. Osteonecrosis of the jaw in cancer after treatment with bisphosphonates: incidence and risk factors. J Clin Oncol. 2005;23:8580–8587.
12. Otto S. Medication-related osteonecrosis of the jaws. e-Book, Springer; 2015.
13. Smith M, Saad F, Coleman R, et al. Denosumab and bone-metastasis-free survival in men with castration-resistant prostate cancer: results of a phase 3, randomised, placebo-controlled trial. Lancet. 2012;379:39–46.
14. Qi W, Tang L, He A, et al. Risk of osteonecrosis of the jaw in cancer patients receiving denosumab: a meta-analysis of seven randomized controlled trials. Int J Clin Oncol. 2014;19:403–410.
15. Graff J, Beer T. Reducing skeletal-related events in metastatic castration-resistant prostate cancer. Oncology (Williston Park, NY). 2015;29:416–423.
16. Kuchuk I, Mazzarello S, Butterfield K, et al. Oral care and the use of bone-targeted agents in patients with metastatic cancers: a practical guide for dental surgeons and oncologists. J Bone Oncol. 2013;2:38–46.
17. Himelstein A, Qin R, Novotny P, et al. CALGB 70604 (Alliance): a randomized phase III study of standard dosing vs. longer interval dosing of zoledronic acid in metastatic cancer. J Clin Oncol. 2015;2015:33. [Abstract 9501].

LETTER TO THE EDITOR

Core needle biopsies for the diagnosis of diffuse large B-cell lymphoma – a great concern for research

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To the Editor,

Core needle biopsies (CNBs) are often the only histopathological material collected at diagnosis or as the disease progresses. These CNBs contain far fewer tumor cells than larger surgical excisional biopsies (SEBs). An illustrative example is the biopsy of lymph nodes that are located deep in the abdominal cavity or mediastinum and, owing to their anatomical position, makes SEBs extremely difficult to perform. Thus, CNBs provide a rapid method for obtaining tumor material and are more commonly the procedure of choice for lymphoma diagnostics [1,2]. That said, according to the World Health Organization classification, CNBs are deemed to be insufficient for diagnostic purposes [3,4]. Instead the guidelines state that material from SEBs should always be obtained to permit a robust histopathological evaluation of the lymph node architecture, growth pattern and cellular composition, and also to ensure that adequate material is available for additional molecular analyses and future research studies.

Tissue microarrays (TMAs) have widely been used in research for the last two decades and enable the simultaneous analyses of selected regions of histological material on a single microscopic slide. The overwhelming benefit of TMA technology is the ability to stain and screen numerous biomarkers within multiple tumors in parallel and at a relatively low cost. The typical tissue cores used for TMA constructs have increased in size from 0.4 mm to 1.6–2 mm in diameter, thereby providing a more comprehensive and representative view of the original architecture of the tumor material and thus save original tissue blocks [5]. In addition, several studies have investigated whether using TMAs to study biomarkers was comparable to using whole tissue sections and the results obtained from both techniques correlated well for all tumor-associated biomarkers [6–8]. Although ongoing efforts are investigating the use of material obtained from CNBs for the construction of TMAs [9], these techniques are not widely applicable and research opportunities are often hindered due to insufficient material. In this study we aimed to use TMAs on a large population-based cohort of diffuse large B-cell lymphoma (DLBCL) patients to assess whether prognostic protein markers were representative for all patients.

Materials and methods

The study population was comprised of patients diagnosed with de novo DLBCL between 2002 and 2012 within the Uppsala Region, Sweden and uniformly treated with the current standard immunochemotherapy regime of rituximab, cyclophosphamide, hydroxydaunorubicine, vincristine and prednisone (R-CHOP). Patients were identified from the Swedish Lymphoma Registry for the Uppsala region and diagnosed at the Department of Pathology at Uppsala University Hospital, which is the referral center for the Uppsala Region. Two-hundred and sixty-five patients were initially identified from the Swedish Lymphoma Registry, which contains approximately 95% of all adult lymphoma patients in Sweden [10]. Patients with a previous history of low-grade B-cell lymphoma (n = 5) were excluded and all tissue material was reexamined and re-classified by an experienced hematopathologist (R.M.A.) before being included in the study. Upon reexamination, an additional eight patients were excluded due to a diagnosis of: (1) T-cell lymphoma (n = 4); (2) Hodgkin lymphoma (n = 3) or (3) lymphoblastic lymphoma (n = 1). No clinical data was available for five patients. Tissue that was representative of the tumor sample and also sufficient in quantity for TMA construction was available for 57 cases, herein referred to as the 'TMA group'. The remaining cases (n = 190) formed the 'non-TMA group'. TMA construction was performed using a manual tissue arrayer (MTA-1; Estigen OÜ Tiigi 61b 50410 Tartu Estonia). In brief, two 1.0-mm tissue cores were taken from areas representative of DLBCL and put in a new recipient block, areas of connective tissue or necrosis were avoided as previously described [8]. Immunohistochemical stainings were performed as previously reported [11].

Clinical data was available for 247 patients. The molecular and clinical characteristics of these patients have previously been reported [11]. Clinical scoring was based on the age adjusted IPI (IPIaa) which defines four risk groups: group 0: low risk; group 1: low–intermediate risk; group 2: intermediate–high risk and group 3: high risk. This study was approved by the Regional Ethical Review Board in Uppsala, Sweden.

Lymphoma-specific survival (LSS) was calculated from the date of diagnosis to the date of death resulting from the lymphoma diagnosis (patients who died from a cause other than lymphoma were censored) or to the date of last follow-up. Overall survival (OS) was calculated from the date of diagnosis to the date of death irrespective of the cause. Survival curves and univariate analyses were performed using the Kaplan-Meier method and the log-rank test was used to compare differences between groups. Contingency tables were analyzed using χ^2 statistics and the level of significance was set at 0.05 using a two-tailed test. All statistical analyses were performed using Statistica 13 software (StatSoft, Scandinavia AB).

Results

A diagnosis of DLBCL was made based on material obtained from CNBs for 156 patients (63%) and from whole tissue sections for the remaining 91 patients (37%). For 124/247 patients, 50% of the study cohort, immunohistochemical staining for BCL-2, BCL-6 and MYC was performed as previously reported [11]. Tumor tissue blocks for TMA construction were available for 57/247 patients (23%) and these patients were noted as having a significantly greater five-year LSS of 92% versus 72% (p = .006) and a five-year OS of 77% versus 62% (p = .05) (Figure 1(a) and (b)). The median follow-up time, when considering all patients, was 53.5 months (range 2.1–118). Patients within the TMA group (n = 57) had a median age at diagnosis of 63 years (range 37–83 years), with a male:female ratio of 2:1. Only 4/57 patients (7%) within the TMA group presented with an IPIaa >2. This was significantly lower compared to the IPIaa

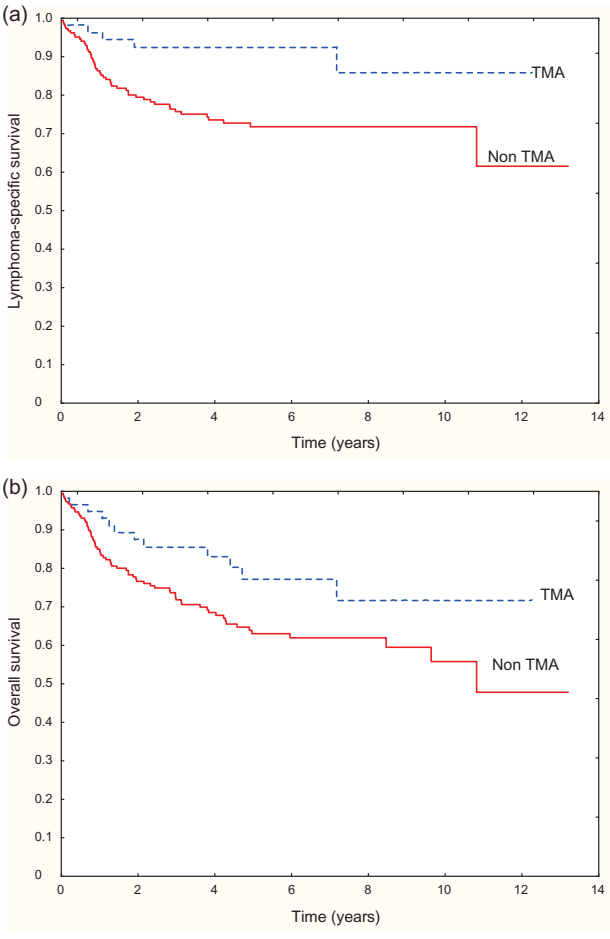

Figure 1. (a) Lymphoma-specific survival for patients assessed by tissue microarray (TMA) (n = 57) and for non-TMA patients (n = 190) (p = .006 log-rank test). (b) Overall survival for patients assessed by TMA (n = 57) and for non-TMA patients (n = 190) (p = .05 log-rank test).

scores of the non-TMA group (n = 190/247) (p = .02). In addition, patients within the TMA group exhibited significant differences in their lactate dehydrogenase (LDH) levels (p = .002). Their tumors had lower expression of BCL-2 (p = .05) or MYC (p < .0001) and dual-expression of BCL-2 and MYC (p = .0003) was also less frequently observed. Overall, the TMA group constituted a patient group with a more favorable prognosis.

Dividing the patients within the TMA group according to disease subtype revealed that 54% (31/57) of patients assessed by TMA had a germinal center (GCB) phenotype whereas the non-germinal center phenotype (non-GCB) was observed in 46% (26/57) of patients (based on the Hans et al. algorithm) [12]. No survival differences were observed between patients assigned to the different subgroups, i.e. GCB versus a non-GCB phenotype. In addition no differences in OS were noted when considering IPIaa score, Ki-67 ≥ 70%, p53 overexpression ≥50%, BCL-2 ≥ 70%, BCL-6 ≥ 30%, MYC ≥40% or dual expression of BCL-2 and MYC was observed; however patient numbers were small.

Discussion

Obtaining sufficient biological material to allow for a complete immunohistochemical analysis, and therefore enable a precise lymphoma diagnosis, is of crucial importance. Although obtaining a correct lymphoma diagnosis is paramount, it would also be highly beneficial if material were available for additional analyses or reevaluation and also for inclusion in future studies or clinical trials. Unfortunately, this scenario is currently not the norm and in our study, due to insufficient material, comprehensive immunohistochemical stainings could only be performed for 50% of the cohort. Rather than being the exception, our study reports figures similar to a previous large clinical study within which adequate material was available for only 52% of patients [13].

For these reasons, the current trend that is emerging regarding the increasing use of CNBs for diagnosis of DLBCL is of great concern [7,8]. Perhaps even more worrying is the fact that CNBs have recently been proposed as the preferred technique for front line diagnostics [1]. Patients with sufficient tumor material for TMA constructions differed from all other DLBCL patients diagnosed during the 11-year study period. Patients within this TMA group had a significantly greater survival and presented with lower IPIaa scores (the vast majority of patients presented with IPIaa ≤2) than patients within the non-TMA group. Tumor markers potentially affecting survival such as the dual expression of MYC and BCL-2, high Ki67 or p53 overexpression were not observed within this TMA group which is in contrast to other studies [14]. However, patient numbers were small and as a consequence no definitive conclusion can be drawn.

Nevertheless, our pivotal observation was that the TMA group consisted of patients with a more favorable outcome. We conclude that these patients are not representative of all DLBCL patients. The consequences for future research studies, particular in the context of larger, collaborative studies where histopathological material (or results) may be shared amongst several centers is of great concern.

Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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References

1. Pruneri G, Gigli F, Rafaniello P, et al. Core needle biopsy as a front line diagnostic approach for lymphoma patients. Hematol Oncol. 2015;33:247–9.
2. Skelton E, Jewison A, Okpaluba C, et al. Image-guided core needle biopsy in the diagnosis of malignant lymphoma. Eur J Surg Oncol 2015;41:852–8.
3. Groneck L, Quaas A, Hallek M, et al. Ultrasound guided core needle biopsies for work-up of lymphadenopathy and lymphoma. Eur J Haematol 2016;97:379–86.
4. Frederiksen JK, Sharma M, Casulo C, et al. Systematic review of the effectiveness of fine-needle aspiration and/or core needle biopsy

for subclassifying lymphoma. Arch Pathol Lab Med 2015;139:245–51.
5. Pedersen MB, Riber-Hansen R, Nielsen PS, et al. Digital pathology for the validation of tissue microarrays in peripheral T-cell lymphomas. Appl Immunohistochem Mol Morphol 2014;22:577–84.
6. Camp RL, Neumeister V, Rimm DL. A decade of tissue microarrays: progress in the discovery and validation of cancer biomarkers. J Clin Oncol 2008;26:5630–7.
7. Aaltonen K, Ahlin C, Amini RM, et al. Reliability of cyclin A assessment on tissue microarrays in breast cancer compared to conventional histological slides. Br J Cancer 2006;94:1697–702.
8. Glimelius I, Qvarnstrom F, Simonsson M, et al. Tissue microarray and digital image analysis: a methodological study with special reference to the microenvironment in Hodgkin lymphoma. Histopathol 2012;61:26–32.
9. Albanghali M, Green A, Rakha E, et al. Construction of tissue microarrays from core needle biopsies – a systematic literature review. Histopathol 2016;68:323–32.
10. Abrahamsson A, Dahle N, Jerkeman M. Marked improvement of overall survival in mantle cell lymphoma: a population based study from the Swedish Lymphoma Registry. Leukemia Lymphoma 2011;52:1929–35.
11. Abdulla M, Laszlo S, Triumf J, et al. A population-based study of cellular markers in R-CHOP treated diffuse large B-cell lymphoma patients. Acta Oncol 2016;55:1126–31.
12. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103:275–82.
13. Salles G, de Jong D, Xie W, et al. Prognostic significance of immunohistochemical biomarkers in diffuse large B-cell lymphoma: a study from the Lunenburg lymphoma biomarker consortium. Blood 2011;117:7070–8.
14. Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012;30:3452–9.

LETTER TO THE EDITOR

Recall radiation myelitis after stereotactic radiation and dabrafenib in metastatic melanoma

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To the Editor,

About 50% of all cutaneous melanomas have an activating mutation in the BRAF gene – 80–90% of those being V600E mutations, resulting in increased proliferation. The BRAF inhibitors like vemurafenib and dabrafenib have resulted in increased overall survival [1]. The interaction with radiotherapy is not yet well explored. We present a case of a possible late radiation recall phenomenon.

A 48-year-old Caucasian woman was diagnosed with metastatic melanoma in 2013. First line treatment was seven courses of nivolumab. In autumn 2014, the patient received four courses of ipilimumab due to progression. A partial response was obtained. Concomitant with ipilimumab the patient received stereotactic radiotherapy in November 2014 to a large central, left-sided pulmonary lung metastasis. Treatment was given with 56 Gy in 8 fractions; 4 fractions per week. The dose to the organs at risk was in concordance with our general practice; the dose to the spinal cord did not exceed 33.5 Gy (Figure 1(a) and (b)). In March 2015, a restaging computed tomography (CT)-scan showed progression. Third line treatment was dabrafenib, initiated March 2015 and with addition of trametinib from April 2015. A follow-up CT-scan showed complete remission by June 2015.

In June 2015, seven months after stereotactic body radiotherapy the patient described a skin rash on her back in the same volume where she received radiotherapy, located below the third thoracic vertebra. The rash was interpreted as a radiation recall phenomenon, caused by dabrafenib. A month later the patient developed back pain in the same site as the rash was seen. A positron emission tomography (PET)-CT showed unspecified activity in the former radiation-treated volume of the spinal cord. Between July and November 2015, the patient's back pain intensified and paresthesias in the lower body and extremities was registered. Magnetic resonance imaging (MRI) showed radiation-induced myelitis in the thoracic spine from TH2 to TH9 (Figure 1(c) and (d)), described as edema. We assume this to be caused by radiotherapy followed by radiosensitization by dabrafenib.

The patient was treated with prednisolone 25 mg/day for one week, tapering off over six weeks. A PET-CT from January 2016 showed no sign of malignant disease in the volume, previously receiving radiotherapy. However, disease progression was found in other sites and dabrafenib and trametinib was reintroduced, without worsening of the myelitis.

In this case, a patient developed radiation recall myelitis after receiving a BRAF inhibitor. Radiosensitization and radiation recall skin toxicity have been reported in relation with treatment with another BRAF inhibitor vemurafenib [2]. Newer case reports describe the same phenomena with dabrafenib [3], but presumably with a lower frequency. To our knowledge this is the first reported case of radiation recall

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