

**Practice point**

The ACRIN trial corroborates the findings of the earlier Department of Defense trial that showed CT colonography to be a safe and valuable screening tool, which effectively ends the validation phase and opens the door for widespread implementation.

positives. The rate of extracolonic findings that would apparently require further attention in this trial was more than double that of other CTC screening experiences,<sup>6</sup> but the methodology was not well described and previously known conditions were not excluded. When extracolonic findings are handled appropriately, CTC has the potential to be both more cost-effective and clinically effective than OC.<sup>7</sup>

Beyond the ACRIN<sup>2</sup> and Department of Defense<sup>1</sup> CTC trials, which have provided the necessary validation albeit in the necessarily artificial setting of a clinical trial, objective evidence now exists that CTC can more efficiently identify patients with advanced neoplasia in actual clinical practice than OC. Kim *et al.*<sup>8</sup> compared parallel CTC and OC screening arms in over 6,000 asymptomatic adults and found that CTC screening yielded the same number of advanced neoplasms (including more cancers) but with fewer total polypectomies (561 versus 2,434), fewer invasive procedures (246 versus 3,163), and fewer complications (none versus seven perforations) compared with primary OC screening.

The cumulative findings of these three studies<sup>1,2,8</sup> demonstrate the high performance of CTC, and led to its inclusion as a recommended screening test in the 2008 revised guidelines of the American Cancer Society.<sup>9</sup> Importantly, these innovative guidelines emphasized the importance of cancer prevention with structural tests that detect precursor polyps over the stool-based tests that primarily detect cancer but are not preventive. By adding another structural screening option that is equally effective but less invasive than OC, overall compliance should increase, resulting in the detection and prevention of more cancers. For the benefit of society, it is time for CTC to move beyond the validation phase and into the realm of widespread implementation. In order to truly effect mortality from colorectal cancer, however, the remaining challenges for this promising test are to achieve widespread acceptance, reimbursement, and implementation.

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**Competing interests**

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1. Pickhardt, P. J. *et al.* Computed tomographic virtual colonoscopy to screen for colorectal neoplasia in asymptomatic adults. *N. Engl. J. Med.* **349**, 2191–2200 (2003).
2. Johnson, C. D. *et al.* Accuracy of CT colonography for detection of large adenomas and cancers. *N. Engl. J. Med.* **359**, 1207–1217 (2008).
3. Pickhardt, P. J., Nugent, P. A., Mysliwiec, P. A., Choi, J. R. & Schindler, W. R. Location of

adenomas missed by optical colonoscopy. *Ann. Intern. Med.* **141**, 352–359 (2004).

4. Pickhardt, P. J. *et al.* Primary 2D versus primary 3D polyp detection at screening CT colonography. *AJR Am. J. Roentgenol.* **189**, 1451–1456 (2007).
5. Pickhardt, P. J., Choi, J. R., Hwang, I. & Schindler, W. R. Nonadenomatous polyps at CT colonography: prevalence, size distribution, and detection rates. *Radiology* **232**, 784–790 (2004).
6. Pickhardt, P. J. *et al.* Unsuspected extracolonic findings at screening CT colonography: clinical and economic impact. *Radiology* **249**, 151–159 (2008).
7. Hassan, C. *et al.* Computed tomographic colonography to screen for colorectal cancer, extracolonic cancer, and aortic aneurysm. *Arch. Intern. Med.* **168**, 696–705 (2008).
8. Kim, D. H. *et al.* CT colonography versus colonoscopy for the detection of advanced neoplasia. *N. Engl. J. Med.* **357**, 1403–1412 (2007).
9. Levin, B. *et al.* Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J. Clin.* **58**, 130–160 (2008).

**HEMATOLOGY****Germinal center or nongerminal center DLBCL?**

Gerhard Held and Michael Pfreundschuh

**Patients with a immunohistochemically defined, germinal-center tumor subtype have an improved outcome compared with patients with the non-germinal-center phenotype when treated with CHOP only, according to a prospective study. The benefit was not observed in those treated with the rituximab combination therapy.**

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid neoplasm. Three subtypes have been identified by gene-expression studies; germinal center (GC) DLBCL, non-GC DLBCL, and mediastinal large B-cell lymphoma. GC and non-GC DLBCL differ with respect to the cell of origin, pathogenetic mechanisms and prognosis. Gene-expression studies require fresh biopsy material or biopsy material that is frozen immediately after excision. Immunohistology of formalin-fixed, paraffin-embedded biopsies using antibodies against CD10, BCL-6 and MUM-1 allows DLBCL subtypes to be categorized to the GC and non-GC subtype.<sup>1</sup>

Many studies have shown that the distinction between the cell origin of GC and non-GC subtypes of DLBCL is an independent prognostic factor for patients

treated with cyclophosphamide, adriamycin, vincristine and prednisone (CHOP) chemotherapy. However, as the addition of rituximab to CHOP (R-CHOP) improved the outcome of all subgroups of patients with CD20<sup>+</sup> DLBCL, a confirmation of the prognostic effect of the cell of origin in DLBCL in the rituximab era is warranted; differences between the subgroups can be assumed to become smaller, which calls in to question the value of the distinction between GC and non-GC DLBCL.

In 2007, a retrospective study in patients with *de novo* DLBCL, who were treated with CHOP or R-CHOP, showed that by assigning the cases to the GC and non-GC subgroups by immunohistochemistry, the classification of DLBCL according to the cell of origin continues to have prognostic importance in the rituximab era. This

analysis in 104 Finnish patients confirmed the improved outcome (that is, improved overall survival and progression-free survival) of patients with immunohistochemically defined GC DLBCL compared with the non-GC phenotype when treated with CHOP only, but this difference was not observed in 90 patients treated with R-CHOP.<sup>2</sup> These results are in contrast to a retrospective, immunohistochemistry-based study conducted in the US in 2008, in which 131 patients with *de novo* DLBCL were treated with R-CHOP, and 112 patients were treated with standard CHOP or CHOP-like therapy only.<sup>3</sup> Addition of rituximab improved the 3-year overall survival (85% versus 52%,  $P < 0.001$ ), and event-free survival (67% versus 47%,  $P = 0.005$ ) of patients with GC DLBCL. Similarly, patients with non-GC DLBCL had improved 3-year overall survival (69% versus 33%,  $P < 0.001$ ), and event-free survival (52% versus 29%,  $P < 0.001$ ) when rituximab was added to CHOP.<sup>3</sup> The use of the Hans classifier for the phenotypic distinction between the GC and non-GC type of DLBCL showed a difference in 3-year overall survival, both for the groups treated with chemotherapy only (52% versus 33%), and also for rituximab-treated patients with GC DLBCL (85% versus 69%,  $P = 0.032$ ).<sup>3</sup>

It is unclear why the Finnish<sup>2</sup> and US<sup>3</sup> studies yielded contrasting results. Both studies are limited by a relatively small number of patients, and both studies were retrospective and used historical controls. To date, there is only one study on patients treated within a randomized trial comparing CHOP with R-CHOP.<sup>4</sup> In the study by Molina and colleagues, multivariate analysis confirmed that only the International Prognostic Index and treatment arm influence the outcome, but not the immunohistochemically defined GC or non-GC phenotype.<sup>4</sup> Unfortunately, of the 399 patients included in the clinical study, immunohistochemistry with tissue microarrays was possible in only 101 patients (25%), which means huge differences in results would be required to reach significance. A small number of patients and historical comparison also limits the validity of a publication from 2008, which

Even if the data were convincing, would it influence our therapeutic strategies?

#### Practice points

- Addition of rituximab to CHOP chemotherapy improves both germinal-center and nongerminal-center diffuse large B-cell lymphoma
- It is unclear whether the immunohistochemical distinction between these subtypes is of prognostic significance in the rituximab setting, and it is currently not relevant for the treatment of these patients

assessed 107 patients with GC DLBCL and 93 patients with non-GC DLBCL, where gene-expression profiling confirmed the prognostic difference of the two groups in response to R-CHOP.<sup>5</sup>

One has to keep in mind that the positive predictive value of the immunohistochemical approach is only 87% for the GC and 73% for the non-GC phenotypes, with a misclassification of 20%.<sup>1</sup> Moreover, the Lunenburg Lymphoma Biomarker Consortium observed highly variable results and poor reproducibility for many markers assessed by immunohistochemistry, and the results did not correlate well for BCL-6, which is included in the Hans classifier.<sup>6</sup>

In summary, none of the studies assessing the prognostic value of the immunohistological phenotype in the rituximab era has sufficient quality to allow for conclusions. Studies assessing tissue microarray data from a larger number of patients, such as the Lunenburg Lymphoma Biomarker Consortium, which includes

patients from different prospective trials,<sup>6</sup> and the German High-Grade Non-Hodgkin Lymphoma Study Group (RICOVER-60),<sup>7</sup> both of which adhere to rigorous protocols for the immunohistochemical procedures, should hopefully address this issue.

Even if the data were convincing, would it influence our therapeutic strategies? We doubt it, because firstly, there is only a small subgroup of patients with such an excellent prognosis that reduction of therapy is justified within prospective trials, such as the ongoing FLYER study of the German High Grade Non-Hodgkin Lymphoma Study Group. This excellent

subgroup of young patients is defined clinically (age <60 years, no risk factor according to the age-adjusted International Prognostic Index, no bulky disease) and not by immunophenotype or gene-expression profiling. Secondly, the outcome of all other patients must be improved, but there are no differential improvement strategies for GC and non-GC DLBCL. With the exception of a subgroup of DLBCL with a stromal-2 signature—which emerged from gene-expression profiling of the stromal cells in DLBCL<sup>5</sup> that might mirror an angiogenic switch, and calls for studies with anti-angiogenic agents such as bevacizumab—no differential therapeutic strategies have emerged to date for DLBCL subgroups. Gene expression and immunohistochemical analyses should be included in randomized trials that are aimed at improving upon R-CHOP treatment, in order to develop differential strategies. Since the studies to date are limited by a small number of patients, the issue of the prognostic effect of the immunophenotypic subclassification of DLBCL remains unsettled and, currently, without therapeutic consequences. For the time being patients with CD20<sup>+</sup> DLBCL who do not qualify for, or do not want to participate in such studies, can be treated with R-CHOP without the knowledge of their cell of origin.

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#### Competing interests

M Pfreundschuh has declared associations with the following companies: Lilly Pharmaceuticals and Roche. See the article online for full details of the relationship. G Held declared no competing interests.

1. Hans, C. P. *et al.* Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* **103**, 275–282 (2004).
2. Nyman, H. *et al.* Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy. *Blood* **109**, 4930–4935 (2007).
3. Fu, K. *et al.* Addition of rituximab to standard chemotherapy improves the survival of both the germinal center B-cell-like and non-germinal center B-cell-like subtypes of diffuse

- large B-cell lymphoma. *J. Clin. Oncol.* **26**, 4587–4594 (2008).
4. Molina, T. J. *et al.* Germinal center phenotype determined by immunohistochemistry on tissue microarray does not correlate with outcome in diffuse large B-cell lymphoma patients treated with immunochemotherapy in the randomized trial LNH98–5. A GELA study [Abstract]. *Blood* **110**, 51 (2007).
  5. Lenz, G. *et al.* Stromal gene signatures in large-B-cell lymphomas. *N. Engl. J. Med.* **359**, 2313–2323 (2008).
  6. de Jong, D. *et al.* Immunohistochemical prognostic markers in diffuse large B-cell lymphoma: validation of tissue microarray as a prerequisite for broad clinical applications—a study from the Lunenburg Lymphoma Biomarker Consortium. *J. Clin. Oncol.* **25**, 805–812 (2007).
  7. Pfreundschuh, M. *et al.* Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20<sup>+</sup> B-cell lymphomas: a randomised controlled trial (RICOVER-60). *Lancet Oncol.* **9**, 105–116 (2008).

## PATHOLOGY

## Are circulating tumor cells predictive of overall survival?

Klaus Pantel and Sabine Riethdorf

**Comparison of circulating tumor cell numbers before and after treatment is predictive for overall survival in patients with metastatic castration-resistant prostate cancer; this comparison is more helpful than prostate-specific antigen detection, according to a recent study.**

Castration-resistant prostate cancer (CRPC) is the second most common cause of cancer-related death in men. As tumor cells in patients with CRPC are able to survive under androgen-depletion, there is an urgent need for the development of alternative targeted therapies. Detection of circulating tumor cells (CTCs) in the blood of patients with CRPC might be of prognostic and predictive relevance and might complement or replace prostate-specific antigen (PSA) determination in predicting and monitoring the response to different therapies.

For patients with CRPC the use of surrogate markers that are easily detected and that define end points for survival after treatment need to be identified. Unfortunately, post-therapy changes in PSA levels do not predict the benefit of long-term treatment for all patients with CRPC.<sup>1</sup> The detection of CTCs in patients with various forms of solid tumors, including prostate cancer, has received considerable attention over the past 10 years.<sup>2</sup> The possibility of taking repeated blood samples for real-time monitoring of the efficacy of systemic therapies opened a new avenue for the development of novel surrogate biomarkers that might improve the clinical management of patients with cancer. In prostate cancer, measurement of cytokeratins and PSA levels are usually used as markers for CTC detection, and the detection rates in patients with CRPC

varied from 38–62% depending on the CTC assay.<sup>3–6</sup>

A study by de Bono *et al.* showed that unfavorable CTCs ( $\geq 5$ ), detected pre-operatively at baseline in 57% of patients with CRPC with the CellSearch<sup>®</sup> (Johnson & Johnson, New Brunswick, NJ) system, was an independent prognostic factor for overall survival.<sup>1</sup> This study showed that the comparison of CTC numbers before and after treatment is predictive for overall survival of these patients and more helpful than PSA detection. The authors described the monitoring of CTCs in the peripheral blood of patients with metastatic CRPC.<sup>1</sup> This prospective study included 231 evaluable patients; unfavorable CTC numbers ( $\geq 5$  CTCs at baseline) were detected in 57% of patients before treatment. Patients were followed up from baseline with regular chart review for up to 36 months from readings drawn for survival analysis. The majority of patients received new first-line chemotherapy (67%), whereas second-line and third-line therapies were applied in 16% and 17% of patients, respectively. While taxotere was used as the chemotherapy in 70% of the patients, 29% of the patients did not receive taxotere, and in 1%

of the patients no data about inclusion of taxotere were available. The key finding was that CTC measurements predicted the clinical outcome (that is, overall survival) of the patients. Remarkably, CTC counts predicted overall survival better than PSA decrement algorithms at all time points. Patients with unfavorable baseline CTC numbers that became favorable (that is,  $\geq 5$  to  $< 5$  CTC numbers per 7.5 ml blood) had an improved overall survival (21.3 months) compared with patients showing unfavorable CTC counts (6.8 months). By contrast, the conversion of a favorable baseline CTC number to an unfavorable CTC number was associated with a worsened prognosis (9.3 months) compared with patients who maintained favorable CTC counts ( $> 26.0$  months).<sup>1</sup> The significance of this study is underlined by the fact that these data have led the FDA to approve this assay for the evaluation of CTCs in patients with CRPC.<sup>1</sup>

The variation in CTC detection rates can be explained, at least in part, by the different detection assays used in studies on patients with CRPC.<sup>3–6</sup> Each assay is associated with its own advantages and limitations. In general, real-time polymerase chain reaction assays for PSA mRNA detection, although very sensitive, provided lower incidences of positive findings than immunocytochemical approaches, such as the CellSearch<sup>®</sup> (Johnson & Johnson) system used in this study<sup>1</sup> and previous studies on patients with CRPC.<sup>3,4</sup> PSA mRNA measured by real-time polymerase chain reaction, however, seems to be a significant and independent prognostic factor for time to progression<sup>5</sup> and for survival,<sup>6</sup> and the results were superior to single serum PSA determination.<sup>6</sup>

The semi-automated CellSearch<sup>®</sup> (Johnson & Johnson) system has gained considerable attention because it allows both standardized, automated immunomagnetic epithelial cell adhesion molecule-based (EpCAM) enrichment, as well as pan-cytokeratin (cytokeratins 8, 18 and 19) staining of CTCs in blood samples. In healthy individuals and patients with benign disease, cells fulfilling the criteria of CTCs were very rarely detected, whereas CTCs were found in patients with metastatic disease from all

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