DNA-cytometric grading of prostate cancer systematic review with descriptive data analysis

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Abstract
Gleason-score ≤6, assessed on core needle biopsies, is an essential prognostic parameter to offer the strategy of Active Surveillance (AS) to patients with locally confined cancers of the prostate. Yet, its interobserver reproducibility is low (48-70%) and its prognostic validity unsatisfactory. An option to complementary assess the malignant potential of these cancers are objective DNA-ploidy-measurements on existing biopsies. For that purpose chromosomal heterogeneity is indirectly quantified by DNA-cytometry resulting in DNA-grades of malignancy 1–4. This review systematically trawls and evaluates all scientific publications on the potential diagnostic and prognostic validity and the heterogeneity of DNA-ploidy measurements in cancers of the prostate between 1966 and 2013. Publications have been classified into Oxford levels of evidence and levels of significance were given for the correlation of DNA-ploidy with different clinical outcomes. 114 scientific articles had to be excluded because of different methodological reasons. All but one of the 67 methodologically acceptable articles report on a significant diagnostic resp. prognostic significance of DNA ploidy measurements in cancers of the prostate. 8 level 1b studies report that DNA-ploidy, assessed on punch biopsies independently predicts organ confinement as assessed after radical prostatectomy. 18 level 2b studies prove that DNA-ploidy measurements add statistically significant information to the Gleason-score. 16 level 2b investigations report a significant correlation of DNA-ploidy with recurrence-free survival. 15 level 2b studies document a significant correlation with overall survival after different types of therapy. 5 level 2b investigations prove a significant correlation with local recurrence or progress after radical prostatectomy. 3 level 2b publications show a significant correlation of DNA-ploidy with the occurrence of lymph node- or bone metastases after radical prostatectomy. 1 level 2b study documents the additional prognostic value of DNA-ploidy measurements over conventional subjective grading in prostate cancer patients under AS. All existing 15 narrative reviews on selected articles dealing with prognostic DNA-cytometry in cancers of the prostate are in favor of this method. Prospective level 1b studies, especially those proving the validity of DNA ploidy measurements to predict non-progression in patients with clinically insignificant low-grade low stage cancers of the prostate eligible for Active Surveillance additionally to the Gleason-score are still missing.

Keywords: DNA-cytometry, DNA-ploidy, DNA-grading, prostate cancer, Gleason-score, active surveillance, brachytherapy, prognosis

Introduction
Epidemiology
Mean age of patients facing the diagnosis of prostate cancer in Germany currently is 70 years. 26.1% of all newly diagnosed malignancies among men are cancers of the prostate. Its incidence has risen from 80 in 1993 to 111.4/100,000 men or 65,830 new cases in 2010. 70,100 new cases are prognosticated for 2014. Nevertheless mortality is constantly decreasing, from 30 in 1993 to 20.0/100,000 men in 2010 [1]. Even lethality is low: 11.7% in the USA in 2006 as compared to other cancers [2]. The favorable five-years survival rate of 93% is mainly due to more frequent early diagnoses as a consequence of PSA-testing [1].
As about 30% of patients who, according to inquest died from prostate cancer, in fact did not according to autopsy [3], the true mortality rates may be significantly lower.

**Therapy**

Adequate therapy of prostate cancers essentially depends on their individual histological type, stage and grade of malignancy. High grades are associated with early and rapid tumor progression and subsequent metastasis. Low grade and low stage cancers may either locally be treated with curative intention (e.g., by radical prostatectomy, external or internal radiation) or subjected to an Active Surveillance (AS) strategy. Different to “Watchful Waiting”, this includes the option for curative therapy if the cancer progresses. About 53% of all newly diagnosed patients with cancers of the prostate in Germany are currently treated by radical prostatectomy, 8% hormonally, 6% by a combination of both, 12% by radiation, 14% by AS and 5% by Watchful Waiting [4].

As the probability of patients with “clinically insignificant microcarcinomas” of the prostate [5] to die from their cancer is very low: 89% overall survival after 8 years [6], 81% overall survival after 10 years [7], the strategy of AS has been designed. About 45% of all screening-detected cancers can be managed with AS [7]. In Germany this strategy is recommended to patients with low-grade Gleason-score(GS)<=6 and low stage (T1c and T2a) cancers, found in <=2 core biopsies with <50% of their volume and a PSA <10 ng/ml. It comprises regular urological examinations, repeated biopsies and PSA-controls but still allows curative therapy if clinical signs of progression can be detected [8].

**Shortcomings of Gleason-grading**

Grading the malignant potential of individual cancers of the prostate currently is performed according to the modified Gleason-score according to the International Society for Urologic Pathology (ISUP) [9] on histological sections of biopsies or resected tissue.

Grading the malignant potential of cancers should be reproducible among different pathologists, representative for the tumor as a whole and, most importantly, prognostically valid. Grading the malignancy of cancers of the prostate should predict outcome of patients even after different types of therapy. Neither the original [10] nor the revised GS [9] reveal sufficient inter-observer reproducibility to rely clinical decisions of the significance of radical prostatectomy vs. AS on this subjective prognostic index only. [11] report a reproducibility of 58–69%, [12] of 48%, [13] of 70% and [14] of 47% for the revised score.

The main cause for the revision of the Gleason-system by the International Society for Urologic Pathology (ISUP) was to enhance its representativity on punch biopsies for the tumor as a whole (as observed in radical prostatectomies). Yet, contrary to what was expected, [15] found an agreement of only 72%.

Two groups furthermore demonstrated that the revised Gleason-grading could neither differentiate the survival of score 7a- and 7b- [16] or GS <=6- and GS7-patients after radical prostatectomy [17].

**Prognostic DNA-cytometry**

Cancers of the prostate, as all other cancers [18] reveal quite different types of chromosomal aneuploidy [19,20]. While malignant tumors progress, their chromosomal sets may become more and more variable, caused by genetic instability [20,21-23]. The resulting “chromosomal chaos” [24,25] can be indirectly quantified by measuring the DNA-content of hundreds to thousands of cancer cells. These methods are called DNA-flow-cytometry [26,27], respectively DNA-image-cytometry [28-34].

It is based on measurements of the Integrated Optical Density (IOD) of stoechiometrically and specifically DNA-stained nuclei and internal calibration with normal, diploid reference cells. Measurements of nuclei under UV-light, previously stained with DNA-specific fluorescent dyes, like DAPI, in liquids flowing through a capillary are called “DNA Flow Cytometry”. Its disadvantage is that the cells are lost after analysis, thus control measurements are not possible. Furthermore cancer cells cannot be differentiated from non-epithelial cells without additional immunocytochemical markers. Measurements on Feulgen-stained nuclei [35] on glass slides, using TV-image-analysis systems are called “DNA Image Cytometry”. It has the advantage that it can repeatedly be performed on prestained and specifically restained slides on individually preclassified cells. Its performance has been highly standardized by a task force of the European Society for Analytical Cellular Pathology, ESACP [30,32-34]. For the purpose of grading the malignant potential of selected solid tumors, four grades of increasing malignancy have been agreed upon: peridiploid (grade 1), peritetraploid (grade 2), x-ploid (grade 3) and multiploid (grade 4) (Table 1 and Figure 1).

Interobserver reproducibility of prognostic DNA-histogram interpretations of prostate cancer biopsies has been reported to be 93.0% and 90.2% [36,37].

**Existing reviews**

14 narrative reviews have so far addressed diagnostic or prog-

| DNA-grade | Definition |
|-----------|------------|
| 1 (Peridiploid, Type A) | One stemline at 2c±10% |
| 2 (Peritetraploid, Type B) | One stemline at 2c±10%, second stemline at 4c±10% |
| 3 (X-ploid, Type C) | One additional stemline outside 1, 8c-2, 2c or 3, 6c-4, 4c±10% |
| 4 (Multiploid, Type D) | More than one stemline outside1, 8c-2, 2c or 3, 6c-4, 4c±10% |
Figure 1. Typical DNA-histograms, corresponding Gleason-scores and tentative prognosis. Frequencies* from [37], prognostic DNA-categories according to [41], 2001.

| Typical DNA-histogram | DNA-grade vs. Gleason-Score | Prognosis Therapy Frequency |
|-----------------------|-----------------------------|-----------------------------|
| **Peridiploid**       | Type A                      | Very good                   |
| **Type A**            | DNA-grade 1                 |                             |
|                      | Correlates about to GS <=6  |                             |
|                      | Active surveillance in locally confined carcinomas |
|                      | In ca. 55% of punch biopsies*|                             |
| **Peritetraploid**    | Type B                      | Still good                  |
| **Type B**            | DNA-grade 2                 |                             |
|                      | Correlates about to GS 7    |                             |
|                      | For elder patients similar as in grade 1 |
|                      | In ca. 26% of punch-biopsies*|                             |
| **X-ploid**           | Type C                      | Not so good                 |
| **Type C**            | DNA-grade 3                 |                             |
|                      | Correlates about to GS 8    |                             |
|                      | Treatment acc. to S3-guidelines |
|                      | In ca. 10% of punch-biopsies*|                             |
| **Multiploid**        | Type D                      | Not good                    |
| **Type D**            | DNA-grade 4                 |                             |
|                      | Correlates about to GS 9&10 |                             |
|                      | Treatment acc. to S3-guidelines |
|                      | In ca. 9% of punch-biopsies*|                             |
nostic DNA-cytometry in cancers of the prostate between 1992 and 2006 [38-51] Table 2. They have reviewed between 2 and 36 publications, mean 12.8. Two of them dealt with DNA-flow cytometry only [30,46]. Besides [46], who did not validate their findings, all of them concluded that this method is of diagnostic or prognostic relevance:

- “Ploidy predicts prognosis significantly” [38].
- “Ploidy looks promising following radical prostatectomy” [39].
- “DNA-ploidy is a CAP (College of American Pathologists) category II method” [40].

Table 2. Reviews dealing with DNA-cytometry in prostate cancer.

| Authors                  | Year | Publications reviewed | Systematic Flow/Image cytometry | Methodological aspects | Prognostic significance | Comparison with other markers |
|--------------------------|------|-----------------------|---------------------------------|------------------------|-------------------------|------------------------------|
| Buhmeida et al., [38]    | 2006 | 14                    | No FCM&ICM                      | Yes                    | “Predicts P significantly in organ confined disease” | Yes N=7                      |
| Montironi et al., [39]   | 2006 | 2                     | No FCM                          | No                     | Not done                | No                           |
| Epstein et al., [40]     | 2005 | 18                    | No FCM&ICM                      | Yes                    | “Ploidy looks promising following RPE”                | Yes N=16                     |
| Ross et al., [41]        | 2003 | 8                     | No FCM&ICM                      | No                     | DNA-ploidy=CAP category II | Yes N=28                    |
| Chakravanti and Zhai et al., [42] | 2003 | 8                     | No FCM&ICM                      | No                     | Predicts P independently | Yes N=29                    |
| Mazzucchetti et al., [43] | 2002 | 8                     | No FCM&ICM                      | No                     | “Provides important prognostic information”           | Yes N=1                     |
| Miller et al., [44]      | 2001 | 6                     | No FCM&ICM                      | No                     | “Questionable independent variable”                   | Yes N=3                     |
| Bostwick et al., [45]    | 2000 | 5                     | No FCM&ICM                      | No                     | DNA-ploidy =CAP category II                           | Yes N=6                     |
| Sakr and Grignon et al., [46] | 1997 | 16                    | No FCM&ICM                      | No                     | “Good potential as prognostic marker”                 | Yes N=3                     |
| Mikuz et al., [47]       | 1997 | 4                     | No FCM&ICM                      | No                     | “Difficult to understand why these well documented data have not yet gained access to treatment protocols” | No                           |
| Schröder et al., [48]    | 1994 | 36                    | No FCM&ICM                      | Yes                    | WHO-consensus conference: “DNA-ploidy is of value in treatment decisions, particularly when surveillance is a treatment option”. “DNA-ploidy should uniformly be studied in clinical trials, particularly in patients with localized cancer”. | No                           |
| Shankey et al., [26]     | 1993 | ?                     | No FCM                          | Yes                    | “Any sample shown to contain representative tumor can provide meaningful information” | No                           |
| Lieber et al., [49]      | 1992 | 12                    | No FCM&ICM                      | No                     | “DNA-diploid tumors have a better prognosis than tumors of a similar stage and grade that are non-diploid” | No                           |
| Deitch et al., [50]      | 1992 | 8                     | No FCM                          | No                     | “FCM has much to tell us about the natural history and biologic behaviour of prostate cancer” | No                           |
| Böcking et al., [51]     | 1992 | 34                    | No FCM&ICM                      | Yes                    | “DNA-cytometry is a powerful tool for grading the malignant potential of prostatic carcinomas, superior to histological and cytological evaluation” | No                           |
patients with localized cancer” [47].

- “In retrospective studies … any sample shown to contain representative tumor can provide meaningful information” [48].
- “DNA-diploid tumors have a better prognosis than tumors of a similar stage and grade that are non-diploid” [49].
- “Flow cytometry has much to tell us about the natural history and biologic behavior of prostate cancer” [50].
- “DNA-cytometry is a powerful tool for grading the malignant potential of prostatic carcinomas, superior to histological and cytological evaluation” [51].

As inclusion of patients with locally confined cancers of the prostate into the strategy of AS requires a valid prognostic assessment of individual tumors and the subjective Gleason-score suffers from low inter-observer reproducibility and insufficient prognostic validity, more reliable prognostic biomarkers are required. So far no systematic review exists on the prognostic validity of DNA-ploidy measurements, that have to be considered to supplement the Gleason-score on identical specimens. This study provides the first systematic review on that subject.

Review

Systematic review of the literature

A query has been performed in PubMed for publications between January 1966 [52] and August 19th, 2013 with the following key words: “prostate cancer and (DNA-ploidy or DNA-aneuploidy or DNA-cytometry or DNA-image-cytometry)”. Studies were classified into different levels of evidence according to their design, applying the criteria of the Oxford Center for Evidence Based Medicine [53]:

- Level 1b, diagnosis: Validating cohort studies with good reference standards or clinical decision rule, tested within one clinical center.
- Level 2b, diagnosis: Exploratory cohort studies with good reference standard or clinical decision rule after derivation or validated on split samples or data bases.
- Level 1b, prognosis: Individual inception cohort studies with >80% follow-up or clinical decision rule, validated in a single population.
- Level 2b, prognosis: Retrospective cohort studies or follow-up of untreated control patients in a randomized controlled clinical trial. Derivation of a clinical decision rule or validated on split samples only.
- Level 3b, prognosis: Retrospective cohort studies with insufficiently defined inclusion criteria or less than 80% of follow-up.

A. B. has performed the review. No reports were excluded because of their status of publication. A systematic assessment of publication bias had not been performed.

The following features were considered as “good reference standards”: For the correlation with diagnosis, the results of histological examination of radical prostatectomies, especially concerning extracapsular spread and infiltration of seminal vesicles. For the correlation with prognosis, the recurrence-free- or overall survival time, the occurrence of lymph node- or bone metastases, clinical proof of local progression or recurrence or a so-called biochemical recurrence.

The **diagnostic accuracy** of specific indices of nuclear DNA distribution obtained on pretherapeutic biopsies, e.g., to render spread beyond the capsule more likely, should be compared with that of the Gleason-score in studies meeting the criteria of Oxford level of evidence 1b. Similarly the **prognostic validity** of indices of nuclear DNA-distribution should be investigated in comparison with the Gleason-Score, specific for different therapeutic settings, in Oxford level of evidence 1b studies.

Excluded papers

1,819 titles had been listed and 1 found through other sources. After exclusion of 40 duplicates and reading the respective abstracts, 1,573 records have been excluded and full texts of 207 publications that seemed to deal with the above mentioned subjects were ordered and reviewed (Figure 2). 114 of these have been excluded from further evaluation due to different types of methodological shortcomings [61-174].

- 32 revealed an inadequate study design: 10 comprised < 50 patients [54-63], 6 had a mixture of different types of therapy [64-69], 5 missed sufficient therapeutic information [70-74], 3 missed sufficient follow-up information [75-77], 4 applied an inadequate gold standard (digital rectal examination, cancer volume) [78-81], 2 selected prognostically extreme groups of patients [82,83], 1 comprised mixed tumor-stages [84], 1 presented no details on recurrence [85].
- 24 correlated DNA-ploidy with non-diagnostic or prognostic features: 5 with morphometry only [86-90], 4 with changes under therapy [91-94], 1 with effects of radiation [95], 2 with stage only [96,97], 2 with cytological grade [98,99], 2 with cancer diagnosis instead of prognosis [100,101], 2 with 5α-reductase [102,103], 1 with PSA and Gleason-score [104], 1 with stage and cytological grade [105], 1 with Gleason-score and stage [106], 1 with histological subtype [107], 1 with stage and non Gleason-grade [108], 1 with steroid receptors [109].
- 25 dealt with methodological aspects of cytometry only [110-134].
- 14 applied an inadequate cytometric methodology: 8 an inadequate sampling of cells [135-142], 3 performed measurements on sections of different thickness [143-145], 1 applied an inadequate internal calibration [146], 1 missed information on cytometric method [147], 1 measured only 30 nuclei per specimen [52].
- 19 various reasons: 7 were not written in English language [148-154], 3 presented case reports [155-157], 2 dealt with rat prostate cancers [158,159], 2 presented no own data [160,161], 1 correlation of biopsy and radical prostatectomy [162], 1 was redundant with a previous
Records identified through database searching (n=1,819)

Records after duplicates removed (n=1,780)

Records screened (n=1,780)

Records excluded (n=1,573)

Full-text articles excluded, with reasons (n=113)

Studies included in qualitative synthesis (n=81)

Studies included in quantitative synthesis (meta-analysis) (n=67)

Figure 2. PRISMA-flow diagram for handling of records (www.prisma-statement.org).

Methodologically sufficient papers
Papers that did not reveal the above mentioned shortcomings were considered as “methodologically sufficient”. 66 publications reported statistically significant correlations between various DNA-ploidy parameters and one of the above-mentioned patient-relevant endpoints. These comprised 15,693 patients (Tables 3-8):

- 8 level 1b studies reported a significant correlation of DNA-cytometric features with histologically proven cancer spread beyond the capsule as detected after radical prostatectomy [167-174]. 4 of them documented a significant improvement of diagnostic accuracy concerning the prediction of organ confinement by DNA-ploidy features over Gleason-score alone (Table 3).
- 10 level 2b studies were found that report on a statistically significant correlation of DNA-cytometric features with recurrence-free survival after radical prostatectomy in a multivariate-analysis [16,175-179,181,184-186], 3 in
an univariate analysis [180,182,183] Table 4. [193] found the same after external radiation in a multivariate analysis. 4 level 3b studies [187-190] proved a significant correlation of DNA-ploidy parameters with recurrence free survival time after radical prostatectomy on multivariate analyses (Table 4).

- 2 level 3b studies [110,169] proved an independent correlation of DNA-ploidy parameters with overall survival time under AS apart from histological or cytological grading in a multivariate design (Table 5). 1 level 2b study did the same multivariate for recurrence free survival time [197] (Table 4).

- 6 level 2b studies proved a significant correlation of DNA-ploidy with overall survival after radical prostatectomy [180,200], 2 of them in a multivariate design [178,198,199,201]. 4 level 3b studies do the same [190,202,203], 1 of them univariate [204]. 6 studies provided a significant correlation of DNA-ploidy with overall survival after hormonal therapy in a multivariate design [169,208-211,213]. 6 level 3b-studies [85], 7 of them multivariate, showed the same [110,169,214-217]. 2 level 3b studies dealt with overall survival after AS [43,64] and report a significant correlation in a multivariate analysis. [212] represents the only publication in which DNA-ploidy did not correlate with survival. But “neither Gleason-score nor WHO-grade correlated” (Table 5).

- 18 level 2b studies report that DNA-ploidy parameters add significant independent prognostic information to the Gleason-score, 12 of them after radical prostatectomy [15,170,175-178,181,185,186,198,222,225] 2 after hormonal therapy [199,208], 1 after external radiation [205] 1 after AS [197], 1 after brachytherapy [207] and 1 after transurethral resection [197]. 9 level 3b studies report the same after radical prostatectomy [167,170,184,187,189,190,203,215,222] Table 6.

- 5 level 2b studies [170,180,182,190,225] report a significant correlation between DNA-ploidy parameters and the occurrence of local progression or recurrence after radical prostatectomy, 1 after hormonal therapy [234], 1 after brachytherapy [227] (Table 7).

- 3 level 2b [170,186,229] and 1 level 3b study [173] report on a significant correlation of DNA-ploidy parameters with the occurrence of lymph node- or bone metastases after radical prostatectomy. 2 level 3b studies report the same after hormonal therapy [224,235] Table 8.

### Tumor heterogeneity

The following publications dealt with aspects of heterogeneity of DNA-ploidy patterns in cancers of the prostate and representativity of punch biopsy for the tumor as a whole.

- Only 3/78 (3.8%) diploid needle-biopsy-DNA-histograms were discrepant to those obtained on subsequent prostatectomy specimens of stage A2-B2 cancers (diploid, aneuploid), while 21.4% of biopsies had been undergraded cancers as Gleason-low-grade [170].

- 141 separate cancer foci had been investigated in 68 radical prostatectomy specimens of different stages of cancer (mean 2.1 per prostate), [39] (n=43) showed heterogeneity of DNA-ploidy pattern (diploid, non-diploid) [171].

- 122 simulated punch biopsies had been investigated from nine prostatectomies containing cancers of unknown stage (mean 12 samples). Five (56%) showed heterogeneity of the DNA pattern (diploid, tetraploid, aneuploid). All four cases having a homogenous DNA content were DNA diploid in all samples. In those cases with a heterogeneous pattern, the areas having abnormal DNA-patterns could not be predicted by histologic pattern or grade [228].

- These authors compared DNA-ploidy patterns (diploid vs. non-diploid) in punch biopsies and subsequent prostatectomy specimens in 12 cases with cancer. Four sections per resected cancer of unknown stage had been investigated. The concordance was to 92% [230].

- Heterogeneity of DNA-ploidy patterns (diploid, tetraploid, aneuploid) had been found in 50% of 39 T2 and T3 cancers

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Table 3. Correlation of DNA-ploidy on biopsies with extracapsular spread (ECS) after radical prostatectomy (RPE). Bold p-values refer to Cox multivariate regression analysis.

| Year (Publication) | Journal | Number of patients investigated | Months follow-up | Significance p | Flow/Image cytometry |
|--------------------|---------|--------------------------------|------------------|---------------|---------------------|
| Oxford level 1b    |         |                                |                  |               |                     |
| Ishaarwal et al., [167] | 2009 J Urol | 370                            | 5                | <0.001        | AUC-ROC+1.5%        |
| Brinker et al., [168] | 1999 J Urol | 159                            | --               | 0.003         | ICM                 |
| Vesalainen et al., [169] | 1994 Br J Cancer | 273                        | 156              | <0.0001       | FCM                 |
| Ross et al., [170] | 1994 Cancer | 89                             | 31.2             | 0.04          | ICM                 |
| Green et al., [171] | 1994 J Urol | 70                             | --               | <0.0001       | ICM                 |
| Häggmann et al., [172] | 1994 Scand J Urol Nephrol | 54                       | --               | <0.0001       | ICM                 |
| Ross et al., [173] | 1994 Mod Pathol | 56                         | 28.8             | 0.03          | ICM                 |
| Badalament et al., [174] | 1991 Cancer | 112                           | --               | 0.04          | FCM                 |

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Table 4. Correlation of DNA-ploidy with recurrence-free survival time. Bold p-values refer to Cox multivariate regression analysis.

| Year | Journal | Number of patients | Months Follow-up | Significance p | Flow/Image cytometry | Comment |
|------|---------|--------------------|------------------|----------------|----------------------|---------|
| **After RPE** | | | | | | |
| **Oxford level 2b** | | | | | | |
| Bantis et al., [175] | 2009 | Tumori | 112 | ¥ 60 | **0.001** | ICM | pT2a-c, pT3a |
| Pretorius et al., [16] | 2009 | Cell Oncol | 186 | ¥ 73.3 | GS 7 <0.001 | ICM | -- |
| Bantis et al., [176] | 2005 | J Exp Clin Cancer Res | 70 | ¥ 60.0 | <0.007 | ICM | -- |
| Deliveliotis et al., [177] | 2003 | World J Urol | 84 | ¥ 45 | **0.0074** | FCM | -- |
| Amling et al., [178] | 1999 | J Urol | 106 | ¥ 120 | **0.002** | FCM | After salvage prostatectomy |
| Gettman et al., [179] | 1999 | Adult Urology | 211 | 60 | <0.001 | FCM | -- |
| Mora et al., [180] | 1999 | Cancer Control | 65 | ¥ 80 | 0.002 | FCM | -- |
| Lerner et al., [181] | 1996 | J Urol | 904 | ¥ 38.4 | p 0.0089 | FCM | pT1, pT2 |
| Zincke et al., [182] | 1992 | Cancer | 370 | ¥ 60 | 0.0008 | FCM | Plus hormonal treatment |
| Wirth et al., [183] | 1991 | Eur Urol | 80 | 120 | 0.0013 | FCM | pT 1-3 |
| Nativ et al., [184] | 1989 | Mayo Clin Proc | 146 | 94.8 | **0.006** | FCM | Stage C n=146 |
| Blute et al., [185] | 1989 | J Urol | 315 | 96 | **0.0004** | FCM | Stages A, B |
| Winkler et al., [186] | 1988 | Mayo Clin Proc | 91 | ¥ 90 | **0.001** | FCM | Low and high GS |
| **Oxford level 3b** | | | | | | |
| Hawkins et al., [187] | 1995 | Urology | 894 | ¥ 100 | **<0.05** | FCM | Partially HAT, & radiation |
| Carmichael et al., [188] | 1995 | J Urol | 112 | ¥ 102 | **<0.034** | FCM | T2, NO, GS <=6 |
| Voges et al., [189] | 1993 | Eur Urol | 85 | ¥ 35 | **<0.005** | FCM | <8 cm & <30% GS 4/5 |
| Montgomery et al., [190] | 1990 | Arch Surg | 261 | 240 | <0.001 | FCM | Stage B |
| Lee et al., [191] | 1988 | J Urol | 88 | 60 | <0.001 | FCM | Interval free of disease |
| **Oxford level 4** | | | | | | |
| Veltri et al., [192] | 1994 | J Cell Biochem | 124 | ¥ 103.2 | 0.008 | ICM | PSA-recurrence |
| **After external radiation** | | | | | | |
| **Oxford level 2b** | | | | | | |
| Centeno et al., [193] | 1994 | Int J Rad Oncol Biol Phys | 70 | 136 | **0.03** | FCM | T1-4, N0, M0 S-Phase |
| **Oxford level 3b** | | | | | | |
| Khoo et al., [194] | 1999 | The Prostate | 42 | ¥ 62 | 0.035 | FCM | -- |
| Pollack et al., [195] | 1994 | Cancer | 76 | ¥ 40 | **0.05** | FCM | -- |
| **After brachytherapy** | | | | | | |
| **Oxford level 3b** | | | | | | |
| Peters-Gee et al., [60] | 1992 | Cancer | 51 | ¥ 52 | <0.05 | ICM | -- |
| **After hormonal therapy** | | | | | | |
| **Oxford level 2b** | | | | | | |
| Stege et al., [196] | 1992 | J Urol | 67 | >24 | 0.01 | FCM | -- |
| **Oxford level 3b** | | | | | | |
| Visakorpi et al., [67] | 1991 | Br J Cancer | 60 | 120 | 0.0103 | FCM | -- |
| **After active surveillance** | | | | | | |
| **Oxford level 2b** | | | | | | |
| Adolfsson et al., [197] | 1990 | J Urol | 146 | ¥ 50 | **0.018** | FCM | Non-Progression. Therapy if progressed |
| Authors | Year | Journal | Number of patients | Months follow-up | Significance p | Flow/Image cytometry | Comment |
|---------|------|---------|--------------------|------------------|----------------|----------------------|---------|
| Ward et al., [198] | 2005 | BJU International | 816 | $\bar{X}\, 123.6$ | 0.008 | FCM | cT3 only |
| Martinez-Jabaloyas et al., [199] | 2004 | Actas Urol Espan | 54 | $\bar{X}\, 120$ | 0.009 | FCM | With bone marrow metastases |
| Amling et al., [178] | 1999 | J Urol | 106 | $\bar{X}\, 120$ | 0.001 | FCM | After external radiation |
| Myers et al., [200] | 1997 | J Urol | 62 | $\bar{X}\, >120$ | 0.0014 | FCM | Plus hormonal treatment |
| Di Silverio et al., [201] | 1996 | Eur Urol | 85 | $\bar{X}\, 35$ | 0.05 | FCM | -- |
| Zincke et al., [182] | 1992 | Cancer | 370 | $\bar{X}\, 60$ | 0.004 | FCM | Plus hormonal treatment |

**Oxford level 3b**

| Authors | Year | Journal | Number of patients | Months follow-up | Significance p | Flow/Image cytometry | Comment |
|---------|------|---------|--------------------|------------------|----------------|----------------------|---------|
| Bratt et al., [202] | 1996 | Urology | 57 | 54-92 | 0.009 | FCM | S-phase fraction |
| Tinari et al., [203] | 1993 | Cancer | 63 | 84 | 0.0044 | FCM | Stages T1-T4 |
| Miller et al., [204] | 1991 | J Urol | 103 | $\bar{X}\, 60$ | <0.001 | FCM | Stage D2 |
| Montgomery et al., [190] | 1990 | Arch Surg | 261 | 240 | <0.0001 | FCM | Stage B |

**After external radiation**

**Oxford level 3b**

| Authors | Year | Journal | Number of patients | Months follow-up | Significance p | Flow/Image cytometry | Comment |
|---------|------|---------|--------------------|------------------|----------------|----------------------|---------|
| Pollack et al., [205] | 2003 | J Clin Oncol | 149 | $\bar{X}\, 96$ | 0.05 | ICM | |
| Song et al., [206] | 1992 | J Urol | 65 | >120 | 0.0001 | ICM | Cancer cause specific survival |

**After brachytherapy**

**Oxford level 2b**

| Authors | Year | Journal | Number of patients | Months follow-up | Significance p | Flow/Image cytometry | Comment |
|---------|------|---------|--------------------|------------------|----------------|----------------------|---------|
| Martínez-Jabaloyas et al., [208] | 2002 | Urology | 127 | >120 | 0.031 | FCM | |
| Pollack et al., [209] | 1997 | Prostate | 33 | $\bar{X}\, 45$ | 0.008 | FCM | |
| Ahlgren et al., [210] | 1997 | Urology | 96 | $\bar{X}\, 176$ | 0.004 | ICM | |
| Forsslund et al., [211] | 1996 | Cancer | 334 | 360 | 0.001 | ICM | |
| Jorgensen et al., [212] | 1995 | Brit J Cancer | 59 | 36 | n. s. | ICM | Neither GS nor WHO-grade correlated |
| Vesalainen et al., [169] | 1994 | Brit J Cancer | 273 | $\bar{X}\, 156$ | 0.058 | FCM | T1-2, M0 |
| Al Abadi et al., [213] | 1992 | Eur Urol | 271 | >= 24 | <0.015 | FCM | T1-4 |

**Oxford level 3b**

| Authors | Year | Journal | Number of patients | Months follow-up | Significance p | Flow/Image cytometry | Comment |
|---------|------|---------|--------------------|------------------|----------------|----------------------|---------|
| Tribukait [110] | 1993 | Eur Urol | 309 | 176 | <0.0001 | ICM | |
| Van den Ouden et al., [214] | 1993 | J Urol | 963 | 96 | 0.023 | FCM | Stages T1-T4 |
| Di Silverio et al., [215] | 1992 | Eur Urol | 80 | $\bar{X}\, 60$ | <0.005 | FCM | Stage A-D |
| Forsslund et al., [216] | 1992 | Cancer | 145 | 276 | <0.001 | ICM | Cytological grade |
| Fordham et al., [217] | 1986 | Br J Surg | 72 | 6-144 | <0.001 | FCM | HT in 73% |

**Oxford level 4**

| Authors | Year | Journal | Number of patients | Months follow-up | Significance p | Flow/Image cytometry | Comment |
|---------|------|---------|--------------------|------------------|----------------|----------------------|---------|
| Miller et al., [204] | 1991 | J Urol | 103 | >60 | <0.001 | FCM | Stage D2 |

**After active surveillance**

**Oxford level 3b**

|Authors | Year | Journal | Number of patients | Months follow-up | Significance p | Flow/Image cytometry | Comment |
|---------|------|---------|--------------------|------------------|----------------|----------------------|---------|
| Vesalainen et al., [169] | 1994 | Brit J Cancer | 106 | $\bar{X}\, 156$ | 0.02 | FCM | T1-2, M0 |
| Tribukait et al., [110] | 1993 | Europ Urol | 287 | $\bar{X}\, 176$ | <0.001 | FCM | FNABs |

**Oxford level 4**

| Authors | Year | Journal | Number of patients | Months follow-up | Significance p | Flow/Image cytometry | Comment |
|---------|------|---------|--------------------|------------------|----------------|----------------------|---------|
| Tribukait et al., [218] | 1991 | Acta Oncol | 125 | 72 | n.n. | FCM | FNABs |

**Oxford level 3b**

**After TUR**

**Oxford level 2b**

| Authors | Year | Journal | Number of patients | Months follow-up | Significance p | Flow/Image cytometry | Comment |
|---------|------|---------|--------------------|------------------|----------------|----------------------|---------|
| Borre et al., [219] | 1998 | Prostate | 120 | $\bar{X}\, 180$ | 0.024 | FCM | 96 WHO low grades only |

Table 5. Correlation of DNA-ploidy with overall survival. Bold p-values refer to Cox multivariate regression analysis.
Table 6. Addition of independent prognostic information to the Gleason-score. Bold p-values refer to Cox multivariate regression analysis.

| Authors | Year | Journal | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Diagnosis/ Prognosis | Comment |
|---------|------|---------|--------------------|--------------------|----------------|----------------------|---------------------|---------|
| **After RPE** | | | | | | | | |
| **Oxford level 2b** | | | | | | | | |
| Bantis et al., [175] | 2009 | Tumori | 112 | $\geq 60$ | 0.001 | ICM | P | pT2a-c, pT3a |
| Pretorius et al., [16] | 2009 | Cell Oncol | 186 | $\geq 73.3$ | $<0.001$ | ICM | GS 7 |
| Ward et al., [198] | 2005 | BJU international | 816 | $\geq 126.6$ | 0.008 | FCM | | pT3 only |
| Bantis et al., [176] | 2005 | J Exp Clin Cancer Res | 70 | | $<0.007$ | ICM | P | |
| Deliveliotis et al., [177] | 2003 | World J Urol | 84 | $\geq 45$ | 0.0074 | FCM | P | -- |
| Amling et al., [178] | 1999 | J Urol | 106 | $\geq 60$ | 0.002 | FCM | | After external radiation |
| Ross et al., [225] | 1999 | Urology | 211 | $\geq 60$ | $<0.001$ | FCM | P | Prediction of external radiation |
| Blute et al., [222] | 1997 | Adult Urology | 2712 | At primary diagnosis | 0.005 | FCM | D | Correlation with positive margins |
| Lerner et al., [181] | 1996 | J Urol | 904 | $\geq 42$ | p 0.0089 | FCM | | pT1, pT2 |
| Ross et al., [170] | 1994 | Cancer | 89 | $\geq 31.2$ | 0.006 | ICM | P | Metastases & recurrences x3 |
| Blute et al., [185] | 1989 | J Urol | 315 | 96 | 0.0004 | FCM | P | Stages A, B |
| Winkler at al., [186] | 1988 | Mayo Clin Proc | 91 | $\geq 90$ | $<0.001$ | FCM | P | Low and high GS |
| **Oxford level 3b** | | | | | | | | |
| Isharwal et al., [176] | 2009 | J Urol | 370 | 3 | AUC-ROC +1,5% | ICM | D | ECS |
| Ross et al., [225] | 1999 | Am J Surg Pathol | 111 | $\geq 27$ | 0.002 | ICM | P | Disease recurrence |
| Di Silverio et al., [215] | 1996 | Europ Urol | 85 | $\geq 35$ | 0.05 | FCM | -- | |
| Hawkins et al., [187] | 1995 | Urology | 894 | $\geq 100$ | $<0.05$ | FCM | P | Partially HT |
| Ross et al., [170] | 1994 | Mod Pathol | 56 | $\geq 28.8$ | 0.0026 | ICM | P | -- |
| Tinari et al., [203] | 1993 | Cancer | 81 | $\geq 84$ | 0.0044 | FCM | P | Stages T1 – T4 |
| Voges et al., [189] | 1993 | Eur Urol | 85 | 70 | 0.001 | FCM | P | Time to recurrence |
| Montgomery et al., [190] | 1990 | Arch Surg | 261 | 240 | 0.001 | FCM | P | Progression & cause specific survival |
| Nativ et al., [184] | 1989 | Mayo Clin Proc | 38 | $\geq 94.8$ | 0.002 | FCM | P | GS low-grade subgroup |
| **After TUR** | | | | | | | | |
| **Oxford level 3b** | | | | | | | | |
| Nielsen et al., [226] | 1993 | APMIS | 79 | 120 | 0.0035 | FCM | P | Grading acc. to Shelley |
| **After external radiation** | | | | | | | | |
| **Oxford level 2b** | | | | | | | | |
| Pollack et al., [205] | 2003 | J Clin Oncol | 149 | 108 | 0.03 | ICM | P | Survival |
| **Oxford level 3b** | | | | | | | | |
| Song et al., [206] | 1992 | J Urol | 65 | $>120$ | $<0.0001$ | ICM | P | Mayo Grade |
| **After brachytherapy** | | | | | | | | |
| **Oxford level 2b** | | | | | | | | |
| Stephensen et al., [207] | 1987 | Cancer Res | 82 | $\geq 91.8$ | 0.0109 | FCM | | Pelvic lymphnode dissection, D1, N+ |
| **Oxford level 3b** | | | | | | | | |
| Peters-Gee et al., [60] | 1992 | Cancer | 51 | $\geq 52$ | $<0.05$ | ICM | | -- |
| **After hormonal therapy** | | | | | | | | |
| **Oxford level 2b** | | | | | | | | |
| Martinez-Jabaloyas et al., [199] | 2004 | Actas Urol Espan | 54 | 120 | 0.009 | ICM | P | All with bone metastases |
| Martinez-Jabaloyas et al., [208] | 2002 | Urology | 127 | $>120$ | 0.031 | FCM | P | -- |
| **Oxford level 3b** | | | | | | | | |
| Pollack et al., [205] | 2003 | J Clin Oncol | 149 | $\geq 96$ | 0.005 | ICM | P | After external radiation |
Continuation of Table 6.

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Diagnosis/Prognosis | Comment                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|----------------------|--------------------------------|
| Ahlgren et al., [210]          | 1997 | Urology          | 96                 | 176                 | 0.0004         | ICM                   | P                    | FNABs                          |
| Forsslund et al., [211]        | 1996 | Cancer           | 334                | 360                 | 0.001          | ICM                   | P                    | FNABs                          |
| Vesalainen et al., [169]       | 1994 | Br J Cancer      | 101                | 156                 | 0.058          | FCM                   | P                    | --                             |
| Di Silverio et al., [201]      | 1992 | Eur Urol         | 80                 | 60                  | <0.05          | FCM                   | P                    | --                             |
| Fordham et al., [217]          | 1986 | Br J Surg        | 72                 | 6-144               | <0.001         | FCM                   | P                    | Ploidy + GS better than GS alone |

After active surveillance
Oxford level 2b

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Diagnosis/Prognosis | Comment                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|----------------------|--------------------------------|
| Adolfsson et al., [197]        | 1990 | J Urol           | 146                | 50                  | 0.018          | FCM                   | Non-Progression       | FNABs. Therapy if progressed  |

After TUR
Oxford level 2b

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Diagnosis/Prognosis | Comment                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|----------------------|--------------------------------|
| Borre et al., [219]            | 1998 | Prostate         | 120                | 180                 | 0.024          | FCM                   | P                    | 96 WHO low grades only         |

Table 7. Correlation of DNA-ploidy with local recurrence or progress. Bold p-values refer to Cox multivariate regression analysis.

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Diagnosis/Prognosis | Comment                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|----------------------|--------------------------------|
| Ross et al., [225]             | 1999 | Am J Surg Pathol | 111                | 27                  | 0.002          | ICM                   | --                   |                                |
| Ross et al., [170]             | 1994 | Cancer           | 89                 | 31.2                | <0.001         | ICM                   | 3 x more frequent    |                                |
| Zincke et al., [182]           | 1992 | Cancer           | 370                | 60                  | <0.0001        | FCM                   | Plus hormonal treatment |                                |
| Montgomery et al., [190]       | 1990 | Arch Surg        | 283                | 112.8               | <0.001         | FCM                   | Stage B              |                                |
| Winkler et al., [186]          | 1988 | Mayo Clin and Foundation | 91 | >60 | <0.0001 | FCM | Stage D1 | |

After hormonal therapy
Oxford level 2b

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Diagnosis/Prognosis | Comment                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|----------------------|--------------------------------|
| Eskelinen et al., [227]        | 1991 | Eur Urol         | 35                 | 187                 | 0.028          | FCM                   | T1/2                 |                                |

After Brachytherapy
Oxford level 2b

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Diagnosis/Prognosis | Comment                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|----------------------|--------------------------------|
| Keyes et al., [223]            | 2013 | In J Rad Oncol Biol Phys | 94 | 90 | 0.011 | ICM | PSA recurrence | |

Table 8. Correlation of DNA-ploidy with occurrence of lymphnode- or bone metastases. Bold p-values refer to Cox multivariate regression analysis.

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Lymph nodes/ Bone | Remarks                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|------------------|--------------------------------|
| Ross et al., [170]             | 1994 | Cancer           | 89                 | 31.2                | 0.006          | ICM                   | --               |                                |
| Ross et al., [229]             | 1993 | Cancer           | 100                | At primary diagnosis | 0.0001        | ICM                   | L & B | 71 after laparotomy |                                |
| Winkler et al., [186]          | 1988 | Mayo Clin Rep    | 91                 | 90                  | <0.0001        | FCM                   | B                   | D1                             |

Oxford level 3b

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Lymph nodes/ Bone | Remarks                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|------------------|--------------------------------|
| Ross et al., [173]             | 1994 | Med Pathol       | 56                 | 28.8                | 0.0026         | ICM                   | L, B              |                                |

Oxford level 4

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Lymph nodes/ Bone | Remarks                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|------------------|--------------------------------|
| Tucci et al., [57]             | 1994 | Brazilian J Med Biol Res | 28 | 50 | 0.03 | ICM | B |                                |

After hormonal therapy
Oxford level 3b

| Authors                        | Year | Journal          | Number of patients | Months of follow-up | Significance p | Flow/Image cytometry | Lymph nodes/ Bone | Remarks                        |
|--------------------------------|------|------------------|--------------------|---------------------|----------------|-----------------------|------------------|--------------------------------|
| Tribukait [235]                | 1993 | Eur Urol         | 309                | 176                 | <0.0001        | FCM                   | --               |                                |
| Eskelinen et al., [227]        | 1991 | Eur Urol         | 91                 | 187                 | 0.0601         | FCM                   | Ln               |                                |
in radical prostatectomies. Five simulated punch biopsies had been taken per specimen. The risk of underestimation decreased from 60% with one biopsy to 5% with five investigated biopsies [231].

- 123 DNA-histograms from 48 men with prostatectomy due to cancers of unknown stage (mean 2.6) had been compared with those of six preoperative biopsies (diploid, non-diploid). In 34 men (71%) DNA-ploidy in prostatectomies was correctly predicted as either diploid or non-diploid on biopsies. Under-estimation occurred mainly when only one or two biopsies were analyzed [232].

Conclusions

All twelve reviews on diagnostic or prognostic DNA-measurements in prostate cancer published so far are merely "narrative" and not systematic ones. Yet, they all conclude that DNA-ploidy is of either diagnostic or of prognostic value, without considering the methodologic quality of addressed papers.

Shortcomings of published papers

The most frequent cause for exclusion of papers (n=32) was an inadequate study design (not enough patients:<50), mixture of different therapies, lacking therapeutic, clinical or follow-up information, selection of patients. In 13 publications DNA-measurements were methodologically insufficient (inadequate sampling or calibration, measurements of sections of different thickness, paucity of cells). Correlation with non-diagnostic or prognostic features (n=25) and dealing with methodological aspects only (n=24) cannot be criticized. Many scientists did not obey existing respective international and interdisciplinary methodological consensus reports [30,32-34], especially concerning problematical types of specimens (sections), missing performance standards (<300 nuclei) and individual prognostic interpretation of data.

Algorithms for DNA-grading of prostate cancer
[52] have been the first to propose an objective alternative for grading prostate cancer malignancy based on DNA-measurements in cancer cells. Our group has published on "DNA-grading of prostatic carcinoma: Prognostic validity and reproducibility" [233]. Up to 1998 no standardized, internationally agreed algorithms existed, on how to derive prognostically different groups from DNA-histograms of prostate cancers. Each author individually defined at least two, up to five different categories. The only common aspect was that they all comprised a DNA-diploid category as the prognostically most favorable one. In 1998 and 2001 the European Society of Analytical Cellular Pathology (ESACP) "Taskforce on Standardization of Diagnostic DNA-Image Cytometry" has published a detailed proposal how to derive four prognostically relevant groups, resp. grades of malignancy, from DNA-measurements of malignant tumors: peridiploid, peritetraploid, x-ploid and multiploid [33,34] (Figure 1 and Table 1). Unfortunately, not many authors have adopted the respective standardized algorithms since then. Thus, their results concerning the prognostic validity of DNA-grading the malignancy of prostate cancer are hardly comparable. Nevertheless, the main, clinically relevant differentiation refers to DNA-diploidy vs. DNA-non diploidy. During tumor progression, peridiploid cancers primarily increase their rate of proliferation [64,234]. Later on during tumor progression, additional peritetraploid clones evolve [235]. Thus, concerning diploidy vs. non-diploidy, it is not relevant which c-value the peridiploid peak exactly has, but if there is a second peak at 4c or elsewhere. According to [210,234] a prognostically relevant proliferation rate >5% can be stated in peridiploid DNA-histograms, supposed a reasonable number of >1000 of nuclei had been measured to obtain representative results [34].

Diagnostic accuracy

The fact that DNA-ploidy-parameters are able to nearly exclude cancer spread beyond the capsule as detected after radical prostatectomy significantly more precise than the Gleason-Score, has been proven in 8 level 1b studies [167-174], Table 3. Thus DNA-ploidy should additionally be taken into consideration, whenever organ confinement is a prerequisite for certain therapeutic strategies, like AS.

Prognostic validity

For untreated patients with early prostate cancer under Active Surveillance the following results have been published:

- Documented for 120 untreated patients in a multivariate level 2b study the significant superior ability of DNA-ploidy over the histological WHO-grade to predict tumor-specific survival time [219].
- Proved in a multivariate level 1b-study with a statistically significant correlation of DNA-ploidy with recurrence-free survival time in 146 untreated patients in comparison with the cytological grade [197,236].
- Proved for 106 untreated patients in a multivariate level 2b study a statistically significant correlation of DNA-ploidy with overall survival time in comparison with the Gleason-score [169].
- Proved for 287 primary untreated patients in a multivariate level 2b study significant correlation with overall survival time in comparison with the cytological grade [235,236].

Brachytherapy is another standard treatment for organ confined prostate cancer. Patients that most likely reveal cancer spread beyond the capsule have to be excluded from this approach. Using core biopsy material, [223] could correctly predict the majority of failures and non-failures, while Gleason-score failed (Figure 3). DNA-diploid patients had a significantly lower rate of disease recurrence as compared with DNA-aneuploid patients. Thus, DNA-grading of prostate cancer malignancy can be used to further specify the inclusion criteria for brachytherapy.

The fact that DNA-ploidy-parameters could prove in 17 retrospective level 2b studies to add significant prognostic
information to the Gleason-score independent from the type of therapy (Table 6) should encourage scientists to conduct studies in order to confirm these findings on a higher level of evidence as this had already been proposed by a WHO-working group [48]. Yet, level of evidence 1b studies, proving independent prognostic validity of DNA-ploidy over Gleason-score to predict non progression of clinically insignificant prostate cancers under Active Surveillance in a prospective setting are still missing. We recommend to perform these.

Heterogeneity
Data on the representativity of DNA-ploidy measurements on biopsies for the cancer as a whole are heterogeneous and depend on the number of samples investigated. While [168,228] and [230] found discrepancies in only 3.8%, 5.0% and 8.0%, [210,232] reported different ploidy-levels in 24.1% and 29.0%. These figures are lower than comparable ones for the Gleason-score (30%:11). Because DNA-ploidy is inhomogeneously distributed within prostate cancers, especially of advanced stages, as histopathological grades are, it is advisable to investigate all cancer foci in biopsies, either separately or pooled.

Why is DNA-cytometry not used more widely?
Some critical comments on this method overlook the enormous technological input that computer science, digital image analysis and informatics have meanwhile contributed to develop this method, becoming a biologically well founded, fast and valid prognostic technology. The fact that the procedure up to the recent development of digital nuclear classifiers has been too laborious and too time consuming and pathologists have not been sufficiently reimbursed, further prohibited its clinical acceptance.

While retrospective studies proving the independent prognostic validity of DNA-ploidy measurements have been published for all main types of treatment modalities of prostate cancers, prospective level 1b studies are still missing. As no other treatment decision in cancers of the prostate is so much dependent from an objective, reproducible and valid prognostication of an individual cancers behavior as Active Surveillance, prospective studies should especially focus on patients under this strategy.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions

| Authors’ contributions                     | AB | MT | MS | JD | SB |
|-------------------------------------------|----|----|----|----|----|
| Research concept and design               | ✓  |    |    |    |    |
| Collection and/or assembly of data        |    | ✓  | ✓  | ✓  | ✓  |
| Data analysis and interpretation          | ✓  |    |    | ✓  |    |
| Writing the article                       | ✓  |    |    |    |    |
| Critical revision of the article          |    |    | ✓  | ✓  | ✓  |
| Final approval of article                 | ✓  |    |    |    |    |
| Statistical analysis                     | ✓  |    |    |    |    |
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Citation:
Böcking A, Tils M, Schramm D, Dietz J and Biesterfeld S. DNA-cytometric grading of prostate cancer systematic review with descriptive data analysis. Pathol Discov. 2014; 2:7.
http://dx.doi.org/10.7243/2052-7896-2-7