DNA-Cytometric Grading of Prostate Cancer -
Systematic Review of the Literature

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Abstract

„Active surveillance“ is an internationally accepted alternative therapeutic strategy for patients with clinically insignificant microcarcinomas of the prostate. Gleason-score (GS) =< 6 in core biopsies represent the most crucial criterion for inclusion. Yet, Interobserver reproducibility of even the updated GS is low (48-70%) and its prognostic validity remains unsatisfactory. An option to complementary and objectively assess the malignant potential of prostatic carcinomas are 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 heterogeneity of DNA-ploidy measurements in cancers of the prostate between 1966 and 2013.

113 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 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 after different types of therapy. 15 level 2b studies document a significant correlation of DNA-ploidy 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. 2 level 2b document the additional prognostic value of DNA-ploidy measurements over conventional subjective grading in prostate cancer patients under active surveillance. All existing 14 unsystematic reviews on selected articles dealing with prognostic DNA-cytometry in cancers of the prostate are in favor of this method. Representativity of DNA-ploidy as assessed on punch biopsies for prostate cancers as a whole are reported to be 71,0 – 96,2%.
Prospective level 1b studies proving the prognostic validity of DNA-ploidy measurements on punch biopsies 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.

Key words

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. 27,2% of all newly diagnosed malignancies among men are cancers of the prostate. Its incidence has risen from 80 in 1993 to 110,9/100.000 men or 63.440 new cases in 2008. 67.700 new cases are prognosticated for 2012. Nevertheless mortality is constantly decreasing, from 30 in 1993 to 20,6/100.000 men in 2008 (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 92% 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 active surveillance strategies. 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 Active Surveillance (AS) and 5% by Watchful Waiting (4).

As the probability of patients with „clinically insignificant microcarcinomas“ of the prostate (5-7) to die from their cancer is very low: 89 % overall survival after 8 years (8), 81% overall survival after 10 years (9), the strategy of „Active Surveillance“ has been designed. About 45% of all screening-detected cancers can be managed with AS (9). In Germany this strategy is restricted to patients with low-grade (Gleason-score <= 6) and low stage (T1c and T2a) cancers, found in <=2 core biopsies with < 50% of their volume and a PSA < 10 ng/ml (10). It comprises regular urological examinations and PSA-controls but still allows curative therapy if clinical signs of progression can be detected.

Shortcomings of Gleason-Grading

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 ofcancers of the prostate should predict outcome of patients even after different types of therapy. We believe that neither the original (11) nor the revised Gleason score (12) reveal sufficient Interobserver reproducibility to rely clinical decisions of the significance of radical prostatectomy vs. active surveillance on this subjective prognostic index only. (13) report a reproducibility of 58-69%, (14) of 48%, (15) of 70% and (16) 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, (13) found an agreement of only 70%.

Several authors furthermore demonstrated that the revised Gleason-grading could neither differentiate the survival of score 7a- and 7b- (17) or GS <=6- and GS7-patients after radical prostatectomy (18).

Prognostic DNA-cytometry

Cancers of the prostate, as all other cancers (19) reveal quite different types of chromosomal aneuploidy (20-22). While malignant tumors progress, their chromosomal sets may become more and more variable, caused by genetic instability (20, 23-25). The resulting „chromosomal chaos“ (26-30) can be indirectly quantified by measuring the DNA-content of hundreds to thousands of cancer cells. This method is called DNA-flow-cytometry (31, 32) respectively DNA-image-cytometry (33-42).

It is based on measurements of the Integrated Optical Density (IOD) in 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 controll measurements are not possible. Furthermore cancer cells cannot be differentiated from non-epithelial cells without additional immunocytochemical markers. Measurements on Feulgen-stained nuclei (43) 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 (37, 39, 40, 42). For the purpose of grading the malignant potential of selected solid tumors, four grades of increasing malignancy have been agreed upon: peridiploid (grade 1), peritertaploid (grade 2), x-ploid (grade 3) and multiploid (grade 4) (tables 1 and 2).

Interobserver reproducibility of prognostic DNA-histogram-interpretations of prostate cancer biopsies has been reported to be 93,0% and 90,2% (44, 45).

Methods

Systematic review of the literature

A query has been performed in PubMed for publications between January 1966 (46) and August 19th, 2013 with the following key words: prostate cancer and DNA-ploidy or DNA-aneuploidy or DNA-cytometry or DNA-image-cytometry.

The following definitions of study types of the Oxford Center for Evidence Based Medicine (47) were applied:

- 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.

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 „validating cohort studies with good reference standard“ (Oxford level of evidence 1b). The *prognostic validity* of indices of nuclear DNA-distribution should be investigated in comparison with the Gleason-

Score, specific for different therapeutic settings, in „individual inception cohort studies with >80% of follow-up or clinical decision rules, validated in a single population“ (Oxford level of evidence 1b).

Review

Excluded papers

1.819 titles had been listed. After reading the respective abstracts, full texts of 208 publications that seemed to deal with the above mentioned subjects were ordered and reviewed. 113 been excluded from further evaluation due to different types of methodological shortcomings (table 3).

- 32 revealed an inadequate study design: 10 comprised < 50 patients, 6 had a mixture of different types of therapy, 5 missed sufficient therapeutic information, 4 missed sufficient follow-up information, 3 applied an inadequate gold standard (DER, cancer volume), 2 selected prognostically extreme groups of patients, 1 comprised mixed tumor-stages, 1 presented no details on recurrence.
- 25 correlated DNA-ploidy with non diagnostic or prognostic features: 5 with morphometry only, 3 with changes under therapy, 2 with effects of radiation, 2 with stage only, 2 with cytological grade, 2 with cancer diagnosis instead of prognosis, 2 with 5α-reductase, 1 with PSA and Gleason-score, 1 with stage and cytological grade, 1 with Gleason-Score and stage, 1 with histological subtype, 1 with stage and non Gleason-grade, 1 with steroid receptors, 1 with tumor volume.
- 24 dealt with methodological aspects of cytometry only.
- 13 applied an inadequate cytometric methodology: 8 an inadequate sampling of cells, 3 performed measurements on sections of different thickness, 1 applied an inadequate internal calibration, 1 missed information on cytometric method.
- 19 various reasons: 7 were not written in English language, 3 presented case reports, 2 dealt with rat prostate cancers, 2 presented no own data, 1 correlation of bipsy and radical prostatectomy, 1 was redundant with a

previous paper, 1 performed an interlaboratory comparison, 1 compared flow- and image cytometry, 1 was obsolete due to a following paper,

Methodologically sufficient papers

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 4-9):

- 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 (48-55). 4 of them document a significant improvement of diagnostic accuracy concerning the prediction of organ confinement by DNA-ploidy features over Gleason-score alone (table 4).

- 11 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 (17, 56-65), 2 in an univariate analysis (66, 67). (68) found the same after external radiation in a multivariate analysis. 4 level 3b studies (69-72) proved a significant correlation of DNA-ploidy parameters with recurrence free survival time on multivariate analyses (table 5).

- 3 level 2b studies (50, 73-75) proved an independent correlation of DNA-ploidy parameters with overall survival time under active surveillance apart from histological or cytological grading in a multivariate design (table 6). 1 level 2b study did the same multivariate for recurrence free survival time (76) (table 5).

- 6 level 2b studies proved a significant correlation of DNA-ploidy with overall survival after radical prostatectomy (66, 77, 78), 2 of them in a multivariate design (61, 79). 4 level 3b studies do the same (72, 80, 81), 1 of them univariate (82). 6 studies provided a significant correlation of DNA-ploidy with overall survival after hormonal therapy in a multivariate design (83-87). 8 level 3b-studies (88), 7 of them multivariate, showed the same (50, 75, 83-87, 89-92). 2 level 3b studies dealt with overall survival after active surveillance (50, 75) and report a significant correlation in a multivariate analysis. (93) represent the only publication in which DNA-ploidy did not correlate with survival. But „neither Gleason-score nor WHO-grade correlated“ (table 6).

- 18 level 2b studies report that DNA-ploidy parameters add significant independent prognostic information to the Gleason-score, 12 of them after radical prostatectomy (17, 51, 56, 58, 65, 66, 94, 95) 2 after hormonal therapy (83, 96), 1 after external radiation (97), 1 after active surveillance (73), 1 after brachytherapy and 1 after brachytherapy (83, 98). 9 level 3b studies report the same after radical prostatectomy (52, 63, 69, 71, 72, 81, 99-101) (table 7).

- 5 level 2b studies (51, 61, 65, 72, 100) report a significant correlation between DNA-ploidy parameters and the occurrence of local progression or recurrence after radical prostatectomy, 1 after hormonal therapy (102), 1 after brachytherapy (98) (table 8).

- 3 level 2b (51, 65, 103) and 1 level 3b study (52) 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 (75, 102) (table 9).

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.

- (104): 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.

- (51): Only 3/78 (3,8%) diploid needle-biopsy-DNA-histograms were discrepant to those obtained on subsequent prostatectomy specimens of stages A2-B2 cancers (diploid, aneuploid), while 21,4% of biopsies had been undergraded cancers as Gleason-low-grade.

- (53): 141 separate cancer foci had been investigated in 68 radical prostatectomy specimens of different stages of cancer (mean 2,1 per prostate), 39 (= 43%) showed heterogeneity of DNA-ploidy pattern (diploid, non-diploid).

- (105): Leung et al. 1994: 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%.

- (85): In 27/112 (24,1%) patients from whom two or more core needle biopsies with cancer could be investigated, a difference in DNA-ploidy pattern (diploid, tetraploid, aneuploid) was seen.

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

- (107): 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.

Reviews

14 unsystematic reviews addressed diagnostic or prognostic DNA-cytometry in cancers of the prostate between 1992 and 2006 (table 10). They have reviewed between 2 and 36 publications, mean 12,8. Two of them dealt with DNA-flow cytometry only. Besides (108), who did not validate their findings, all of them concluded that this method is of diagnostic or prognostic relevance:

- (109): „Ploidy predicts prognosis significantly“.
- (12): „Ploidy looks promising following radical prostatectomy.
- (110): „DNA-ploidy is a CAP (College of American Pathologists) category II method“.
- (111): „Ploidy predicts prognosis independently“.
- (112): „Ploidy provides important prognostic information“.
- (113): „Ploidy is a questionable independent variable“.
- (114): „DNA-ploidy is a CAP category II method“.
- (115): „DNA-ploidy has good potential as prognostic marker“.
- (116): „It is difficult to understand why these well documented data have not yet gained access to treatment protocols“.
- (117): „DNA-ploidy is of value in treatment decisions, particularly when surveillance is an option“. „DNA-ploidy should uniformly be studied in clinical trials, particularly in patients with localized cancer.
- (31): “In retrospective studies … any sample shown to contain representative tumor can provide meaningful information”.
- (118): „DNA-diploid tumors have a better prognosis than tumors of a similar stage and grade that are non-diploid“.
- (119): „Flow cytometry has much to tell us about the natural history and biologic behavior of prostate cancer“.

- (120): „DNA-cytometry is a powerful tool for grading the malignant potential of prostatic carcinomas, superior to histological and cytological evaluation“.
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Conclusions

Shortcomings of published papers

The most frequent cause for exclusion of papers (n=32) was an inadequate study design (not enough patients, 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 (table 3). Many scientists did not obey existing respective international and interdisciplinary methodological consensus reports (32, 37, 39, 40, 42), 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

(121) 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 (122). 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 (41, 42) (tables 1 and 2). 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 (85, 123). Later on during tumor progression, additional, peritetraploid clones evolve (74). 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 (85, 123) a prognostically relevant proliferation rate > 5% can be stated in peridiploid DNA-histograms, supposed a reasonable number of nuclei of > 1000 had been measured to obtain representative results (40).

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 alone, has been proven in 8 level 1b studies (49, 50-55, 99). Thus DNA-ploidy should additionally be taken into consideration, whenever organ confinement is a prerequisite for certain therapeutic strategies, like active surveillance.

Prognostic validity

For untreated patients with early prostate cancer under active surveillance the following results have been published:

- (73) 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.
- (76) 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 (124).
- (50) 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.
- (75) proved for 287 primary untreated patients in a multivariate level 2b study significant correlation with overall survival time in comparison with the cytological grade (Esposti, 1971).

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, (98) could correctly presict the majority of failures and non-failures, while Gleason-Score failed (figure 1). 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 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 (117). 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 (51), (105) and (106) found discrepancies in only 3,8%, 8,0% and 5,0%, (85) and (107) reported different ploidy-levels in 24,1% and 29,0%. These figures are lower than comparable ones for the Gleason-score (30%: 13). Because DNA-ploidy is inhomogenously distributed within prostate cancers, especially of advanced stages, as hostopathological grades are, it is advisable to investigate all cancer foci in biopsies, either separately or pooled.

Why is DNA-Cytometry not used more widely?

Prognostic DNA-cytometry recently has been addressed as an „old fashioned“ or „outdated“ method, as first publications appeared in the 60ies of the last century. This valuation overlooks the enormous technological input 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. The fact that pathologists are used to subjectively assess cytological or histological images instead of measuring certain features in cells or tissues still prohibits the acceptance of DNA-cytometry in this discipline.

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.

We recommend the implementation of an interdisciplinary uro-pathologic task force on “Prognostic DNA-cytometry in prostate cancer” within an international scientific society of urology or pathology. This group should define useful indications and suitable materials, propose required studies and standardize methods including prognostic interpretations of cytometric data.

Competing interests

A. Böcking has codeveloped a device for DNA-image cytometry together with Prof. Dietrich Meyer-Ebrecht and Dipl. Math. David Friedrich, Institute of Image Analysis and Computer Vision, Aachen University of Technology, Germany and Motic Company in Xiamen, P.R. China.

Author’s contributions

AB performed the analysis and drafted the manuscript, MT and MS performed the query, SB helped drafting the manuscript.

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Tables

Table 1

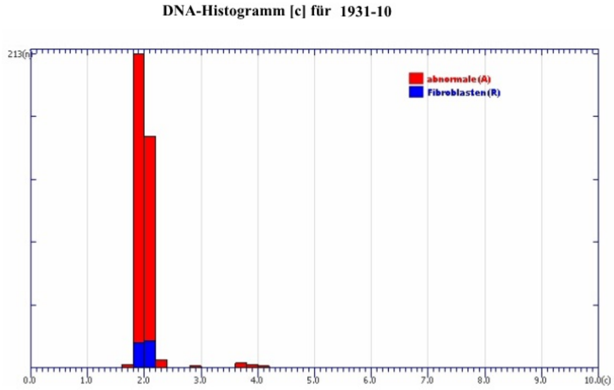
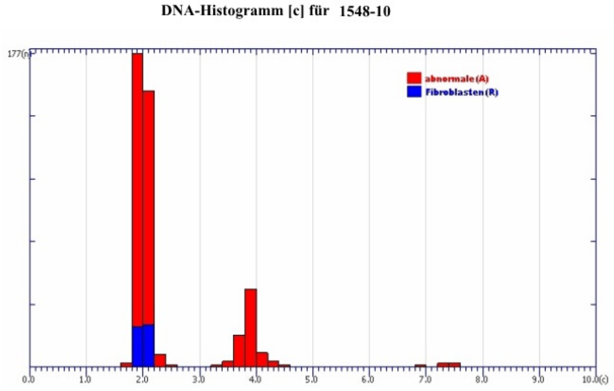
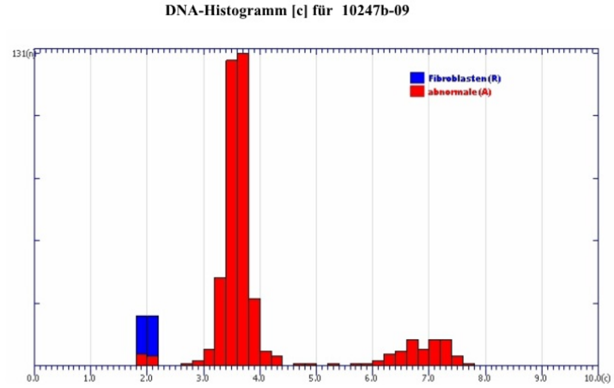
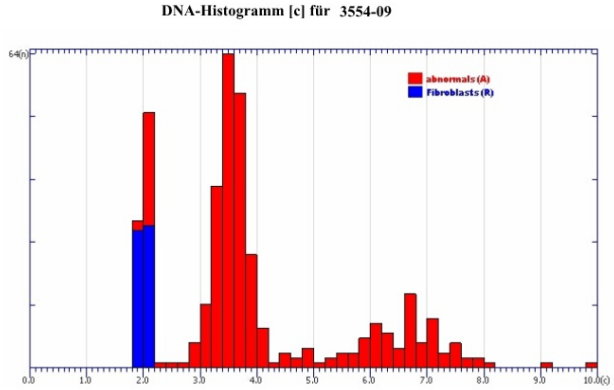
Algorithms for DNA-grading prostate cancer malignancy in four groups (Haroske et al., 1998, 2001)

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 outside 1,8c-2,2c or 3,6c-4,4c +- 10%

Table 2

Typical DNA-histograms, corresponding Gleason-scores and tentative prognosis
* from Tils, 2013; prognostic DNA-categories according to Haroske et al., 1998, 2001.

Autors, year, (# of reference)	Cause of exclusion

Typical DNA-histogram	**DNA-grade vs. Gleason-Score**	**Prognosis Therapy Frequency**
	Peridiploid DNA-grade 1 corresponds about to GS <= 6	Very good Active surveillance in microcarcinomas In ca. 55% of cases*
	Peritetraploid DNA-grade 2 corresponds about to GS 7	Still good For elder patients similar as in grade 1 In about 25% of cases*
	X-ploid DNA-grade 3 corresponds about to GS 8	Rather worse Treatment as with GS >= 8 In about 10% of cases*
	Multiploid DNA-grade 4 corresponds about to GS 9 & 10	Bad Treatment as with GS >=8 In about 10% of cases*

Lee et al., 2012 (125)	Correlation wit 5**α-reductase**
Qian et al., 2010 (126)	Cancer „volume“ as gold standard
Micent et al., 2007 (127)	Article in french
Sengupta et al., 2006 (128)	Measurements on sections of different thickness
Abaza et al., 2006 (129)	< 50 patients
Krause et al., 2005 (130)	Inadequate macroscopic sampling
Lorenzato et al., 2004 (131)	Digital rectal examination as gold standard
Bahn et al., 2004 (132)	„Scrapes“ from unknown number of biopsies
DiMarco et al., 2003 (133)	Measurement on sections of different thickness
Gundorova et al., 2003 (134)	Article in russian
Buhmeida et al., 2002 (135)	No adequate gold standard
Martinez-Jablanoyas et al., 2001 (136)	Article in spanish
Sebo et al., 2001 (137)	Measurements on sections of different thickness
Danielsen et al., 2000 (138)	Article in norwegian
Ahlgren et al., 1999 (139)	Three different types of therapy
Buhmeida et al., 1999 (140)	Different samling techniques
Seay et al., 1998 (141)	Missing information on cytometric method
Gettman et al., 1998 (142)	Obsolete by following paper
Coetzee et al., 1997 (143)	Touch preps from RPEs
Kugler et al., 1997 (144)	Halfs of all biopsies measured by FCM irrespective of cancer content
Moussa et al., 1997 (145)	< 50 patients
Azúa et al., 1997 (146)	No type of therapy, no details of folllow-up
Loo et al., 1996 (147)	Correlation with diagnosis only
Azúa et al., 1996 (148)	No type of therapy, no details of follow-up
Al-Abadi u. Nagel, 1995 (149)	No type of therapy, no details of follow-up
Romics et al., 1995 (150)	< 50 patients
Paz-Bouza et al., 1994 (151)	No clinical- or follow-up data
Müller et al., 1994 (152)	No details on recurrence
Tucci et al., 1994 (153)	< 50 patients
Takai et al., 1994 (154)	Comparison of FCM with ICM
Hussain et al., 1993 (155)	Correlation with stagg
Konchuba et al., 1993 (156)	Sampling method
Babiarz et al., 1993 (157)	< 50 patients
Tribukait, 1993 (75)	Methodology only
Sassi et al., 1993 (158)	Correlation biopsy versus RPE
Ishikawa, 1992 (159)	Article in japanese
Falkmer, 1992 (160)	Methodology only
OSullivan et al., 1992 (161)	Effect of radiation therapy
Fossa et al., 1992 (162)	Interlaborartory comparison
Furusato et al., 1992 (163)	Insufficient follow-up
Montironi et al., 1992 (164)	Correlation with nuclear morphometry
Waehre et al., 1992 (165)	Change under iodine implantation
Yokogi et al. 1991 (166)	< 50 cases
Robertson and Paulson, 1991 (167)	No own data

Nordgren et al., 1991 (168)	Mixed therapies
Wang et al., 1992 (169)	Correlation with nuclear morhometry
Peters Gee et al., 1992 (170)	Mixed therapies
Humphrey et al., 1991 (171)	No information on follow-up; non representative material
Visacorpi et al., 1991 (172)	Mixed therapies
Sahin et al., 1991 (173)	Case report, sarcoma
Nagel and Al Abadi, 1991 (174)	Redundant with following paper 1992
Adolfsson and Tribukait, 1991 (175)	Mixed stages
Falkmer, 1991 (176)	Methodology only
Nativ and Lieber, 1991 (177)	No own data
Piaton et al., 1991 (178)	Article in french
Stekvist and Browen, 1991 (179)	Methodology only
Piaton et al., 1991 (180)	Article in french
Greene et al., 1991 (181)	Correlation with tumor volume
Haugen and Mjolnerod, 1990 (182)	Mixed therapies
Zetterberg and Forsslund, 1990 (183)	Two extreme groups selected
Jones et al., 1990 (184)	Up to 75% normal tissue included
Epstein et al., 1990 (185)	Correlation with nuclear morphometry
Benson et al., 1990 (186)	Correlation with cytologic grades
Ring et al., 1990 (187)	Inadequate internal calibration
Al Abadi and Nagel, 1990 (188)	No information on therapy
Montironi et al., 1990 (189)	Correlation with nuclear morphometry
Nativ et al., 1990 (190)	Correlation with PSA and Gleason-score
Forsslund and Zetterberg, 1990 (191)	Extreme survival groups selected
LeRich et al., 1989 (192)	Correlation with stage and cytological grade
Bibbo et al., 1989 (193)	Correlation with nuclear morphometry
Habib et al., 1989 (194)	Correlation with 5α-reductase
De Vere White and Deitch, 1989 (195)	Diagnosis instead of prognosis
Amberson and Koss, 1989 (196)	Correlation with stage
Benson et al., 1989 (197)	Methodology only
Coon and Weinstein, 1989 (198)	Methodology only
Dejter et al., 1989 (199)	Correlation with Gleason score and stage
Freudenberg et al., 1989 (200)	Methodology only
Howell and Teplitz, 1989 (201)	Case report
Leistenschneider, 1989 (202)	Case reports
Al Abadi and Nagel, 1988 (203)	No information on therapy
Currin et al., 1988 (204)	Correlation with histologic subtype
Klein et al., 1988 (205)	Methodology only
Lundgren et al., 1988 (206)	Rat prostae cancer
Borgman et al., 1988 (207)	Mixed therapies, response to therapy
Ritchie et al., 2988 (208)	No cancer-specific selection of tissue
McIntire et al., 1988 (209)	< 50 patients
Willumsen et al., 1988 (210)	Correlation with stage and non-Gleason-grade
Lundberg et al., 1987 (211)	< 50 patients only
Benson and Walsh, 1986 (212)	Methodology only
Seppelt et al., 1986 (213)	Methodology only
Frankfurt et al., 1985 (214)	< 50 patients
Böcking et al., 1985 (215)	< 50 patients

Schultz et al., 1985 (216)	Methodology only
Auer and Zetterberg, 1984 (217)	Methodology only
Müntzing, 1983 (218)	Methodoogy only
Leistenschneider und Nagel, 1983 (219)	Methodology only
Tribukait et al., 1983 (220)	Correlation with cytological grade
Zimmermann et al., 1983 (221)	Methodology only
Ekman et al., 1981 (222)	Correlation with steroid receptor
Lämmel et al., 1981 (223)	Methodology only
Ronström et al., 1981 (224)	Methodology only
Collins et al., 1981 (225)	Rat prostate cancer
Leistenschneider and Nagel, 1980 (226)	Change under therapy
Tribukait et al., 1980 (227)	Methodology only
Leistenscneider and Nagel, 1979 (228)	Change under therapy
Kjaer et al., 1979 (229)	Change under hormonal treatment
Frederiksen et al., 1978 (230)	Methodology only
Zimmermann and Truss, 1978 (231)	Methodology only
Bichel et al., 1977 (232)	Methodology only
Görttler et al., 1977 (233)	Methodology only
Sprenger et al., 1976 (234)	Methodology only
Zetterberg and Esposti, 1976 (235)	Methodology only
Sprenger at al., 1974 (236)	Methodology only

Table 3

List of excluded publicatios with causes

Figures

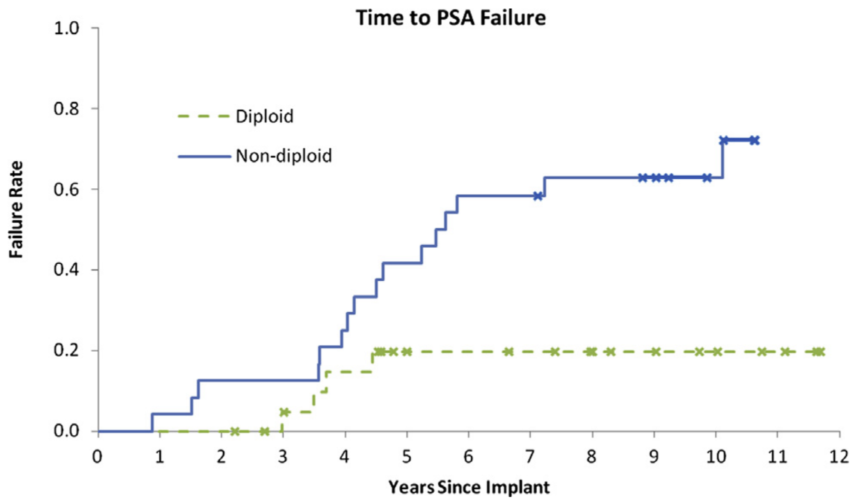


Figure 1

Occurrence of PSA-progress as early indicator of metastasis or recurrence in patients with DNA-diploid and non-diploid cancers of the prostate after brachytherapy. Both groups had the same distribution of Gleason-scores (from; Keyes et al., 2013).

Description of additional data files

Tables 4 – 10 as separate files

Authors	Year	Journal	Number of Patients investigated	Months Follow-up	Significance p	Flow / Image Cytometry
Oxford level 1b						
Isharwal et al. (48)	2009	J Urol	370	5	< 0,001 AUC-ROC + 1,5%	ICM
Brinker et al. (49)	1999	J Urol	159	-	**0,003**	ICM
Vesalainen et al. (50)	1994	Br J Cancer	273	x̄ 156	**< 0,0001**	FCM
Ross et al. (51)	1994	Cancer	89	x̄ 31,2	**0,04**	ICM
Green et al. (53)	1994	J Urol	70	-	< 0,0001	ICM
Häggmann et al. (54)	1994	Scand J Urol Nephrol	54	-	< 0,0001	ICM
Ross et al. (52)	1994	Mod Pathol	56	x̄ 28,8	**0,03**	ICM
Badalament et al. (55)	1991	Cancer	112	-	0,04	FCM

Table 4:

Correlation of DNA-ploidy on biopsies with extracapsular spread (ECS) after radical prostatectomy (RPE).

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. (56)	2009	Tumori	112	x̄ 60	**0,001**	ICM	pT2a-c, pT3a
Pretorius et al. (17)	2009	Cell Oncol	186	x̄ 73,3	**GS 7 < 0,001**	ICM	
Bantis et al. (57)	2005	J Exp Clin Cancer Res	70	x̄ 60,0	**< 0,007**	ICM	
Deliveliotis et al. (58)	2003	World J Urol	84	x̄ 45	**0,0074**	FCM	
Amling et al. (68)	1999	J Urol	106	x̄ 120	**0,002**	FCM	After salvage prostatectomy
Gettman et al. (59	1999	Adult Urology	211	60	**< 0,001**	FCM	
Mora et al. (67)	1999	Cancer Control	65	x̄ 80	0,002	FCM	
Lerner et al. (60)	1996	J Urol	904	x̄ 38,4	**p 0,0089**	FCM	pT1, pT2
Zincke et al. (61)	1992	Cancer	370	x̄ 60	0,0008	FCM	Plus hormonal treatment
Wirth et al. (62)	1991	Eur Urol	80	120	0,00013	FCM	pT 1-3
Nativ et al. (63)	1989	Mayo Clin Proc	146	94,8	**0,006**	FCM	Stage C n=146
Blute et al. (64)	1989	J Urol	315	96	**0,0004**	FCM	Stages A, B
Winkler et al. (65)	1988	Mayo Clin Proc	91	x̄ 90	**0,001**	FCM	Low and high GS
Oxford level 3b							
Hawkins et al. (69)	1995	Urology	894	x̄ 100	**< 0,05**	FCM	Partially HAT, & radiation
Carmichael et al.	1995	J Urol	112	x̄ 102	**< 0,034**	FCM	T2, NO, GS <=6

(70)							
Voges et al. (71)	1993	Eur Urol	85	x̄ 35	**< 0,005**	FCM	< 8 ccm & <30% GS 4/5
Montgomery et al. (72)	1990	Arch Surg	261	240	**< 0,001**	FCM	Stage B
Lee et al. (125)	1988	J Urol	88	60	< 0,001	FCM	Interval fee of disease
Oxford level 4							
Veltri et al. (237)	1994	J Cell Biochem	124	x̄ 103,2	0,008	ICM	PSA-recurrence
After external radiation							
Oxford level 2b							
Centeno et al. (68)	1994	Int J Rad Oncol Biol Phys	70	136	**0,03**	FCM	T1-4, N0, M0 S-Phase
Oxford level 3b							
Khoo et al. (238)	1999	The Prostate	42	x̄ 62	0,035	FCM	
Pollack et al. (239)	1994	Cancer	76	x̄ 40	**0,05**	FCM	
After brachytherapy							
Oxford level 3b							
Peters-Gee et al. (170)	1992	Cancer	51	x̄ 52	< 0,05	ICM	
After hormonal therapy							
Oxford level 2b							
Stege et al. (87)	1992	J Urol	67	> 24	0,01	FCM	
Oxford level 3b							
Visakorpi et al. (172)	1991	Br J Cancer	60	120	0,0103	FCM	
After active surveillance							
Oxford level 2b							
Adolfson et al.	1990	J Urol	146	x̄ 50	**0,018**	FCM	Non-Progression.

(76)							Therapy if progressed

Table 5:

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

Authors	Year	Journal	Number of patients	Months follow-up	Significance p	Flow- / Image Cytometry	Comment
After RPE							
Oxford level 2b							
Ward et al. (77)	2005	BJU International	816	x̄ 123,6	**0,008**	FCM	cT3 ony
Martinez – Jabaloyas et al. (96)	2004	Actas Urol Espan	54	x̄ 120	**0,009**	FCM	With bone marrow metastases
Amling et al. (66)	1999	J Urol	106	x̄ 120	**0,001**	FCM	After external radiation
Myers et al. (79)	1997	J Urol	62	x̄ > 120	0,0014	FCM	Plus hormonal treatment
Di Silverio et al. (101)	1996	Europ Urol	85	x̄ 35	**0,05**	FCM	
Zincke et al. (61)	1992	Cancer	370	x̄ 60	0,004	FCM	Plus hormonal treatment
Oxford level 3b							
Bratt et al. (80)	1996	Urology	57	54-92	**0,009**	FCM	S-phase fraction
Tinari et al. (81)	1993	Cancer	63	84	**0,0044**	FCM	Stages T1-T4
Miller et al. (82)	1991	J Urol	103	x̄ 60	< 0,001	FCM	Stage D2
Montgomery et al. (72)	1990	Arch Surg	261	240	**< 0,0001**	FCM	Stage B
After external radiation							
Oxford level 3b							
Pollack et al. (97)	2003	J Clin Oncol	149	x̄ 96	**0,05**	ICM	
Song et al. (240)	1992	J Urol	65	> 120	**0,0001**	ICM	Cancer cause specific survival
After brachytherapy							
Oxford level 2b							
Stephenson et al. (95)	1987	Cancer Res	82	60-180	**0,0109**	FCM	D1, N1, measured on lymphnodes
After hormonal therapy							
Oxford level 2b							
Martinez-Jablonayas et al. (83)	2002	Urology	127	> 120	**0,031**	FCM	
Pollack et al.	1997	Prostate	33	x̄ 45	**0,008**	FCM	

(84)							
Ahlgren et al. (85)	1997	Urology	96	x̄ 176	**0,004**	ICM	
Forsslund et al. (86)	1996	Cancer	334	360	**0,001**	ICM	
Jörgensen et al. (93)	1995	Brit J Cancer	59	36	**n. s.**	ICM	Neither GS nor WHO-grade correlated
Vesalainen et al. (50)	1994	Brit J Cancer	273	x̄ 156	**0,058**	FCM	T1, M0
Stege et al. (87)	1992	Europ Urol	271	>= 24	**<0,015**	FCM	T1-4
Oxford level 3b							
Pollack et al. (84)	1997	Prostate	33	x̄ 45	**0,008**	FCM	
Vesalainen et al. (50)	1994	Brit J Cancer	101	x̄ 156	**0,058**	FCM	T1-2, M0
Tribukait (75)	1993	Eur Urol	309	176	**< 0,0001**	ICM	
Van den Ouden et al. (89)	1993	J Urol	963	96	**0,023**	FCM	Stages T1 - T4
Al-Abadi and Nagel (88)	1992	Europ Urol	271	120	0,001	ICM	Stages T3 – T4
Di Silverio et al. (90)	1992	Eur Urol	80	x̄ 60	**< 0,005**	FCM	Stage A - D
Forsslund et al. (91)	1992	Cancer	145	276	**< 0,001**	ICM	Cytological grade
Fordham et al. (92)	1986	Br J Surg	72	6-144	**< 0,001**	FCM	HT in 73%
Oxford level 4							
Miller et al. (82)	1991	J Urol	103	> 60	< 0,001	FCM	Stage D2
After active surveillance							
Oxford level 3b							
Vesalainen et al. (50)	1994	Brit J Cancer	106	x̄ 156	**0,0058**	FCM	T1-2, M0

Tribukait (75)	1993	Europ Urol	287	x̄ 176	**< 0,001**	FCM	FNABs
Oxford level 4							
Tribukait (74)	1991	Acta Oncol	125	**72**	**n.n.**	FCM	FNABs
Oxford level 3b							
Nach TUR							
Oxford level 2b							
Borre et al. (73)	1998	Prostate	120	x̄ 180	**0,024**	FCM	96 WHO low grades only

Table 6:

Correlation of DNA-ploidy with overall survival. 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. (56)	2009	Tumori	112	x̄ 60	**0,001**	ICM	P	pT2a-c, pT3a
Pretorius et al. (17)	2009	Cell Oncol	186	x̄ 73,3	**< 0,001**	ICM		GS 7
Ward et al. (77)	2005	BJU international	816	x̄ 126,6	**0,008**	FCM		pT3 only
Bantis et al. (56)	2005	J Exp Clin Cancer Res	70	x̄ 60	**< 0,007**	ICM	P	
Deliveliotis et al. (58)	2003	World J Urol	84	x̄ 45	**0,0074**	FCM	P	
Amling et al. (66)	1999	J Urol	106	120	**0,002**	FCM		After external radiation
Ross et al. (49)	1999	Urology	211	60	**< 0,001**	FCM	P	Prediction of recurrence
Blute et al. (94)	1997	Adult Urology	2712	At primary diagnosis	**0,005**	FCM	D	Correlation with positive margins
Lerner et al. (60)	1996	J Urol	904	x̄ 42	**p 0,0089**	FCM		pT1, pT2
Ross et al. (51)	1994	Cancer	89	x̄ 31,2	**0,006**	ICM	P	Metastases & recurrences x3
Blute et al. (64)	1989	J Urol	315	96	**0,0004**	FCM	P	Stages A, B
Winkler at al. (65)	1988	Mayo Clin Proc	91	x̄ 90	**<0,001**	FCM	P	Low and high GS
Oxford level 3b								
Isharwal et al. (48)	2009	J Urol	370	3	AUC-ROC + 1,5%	ICM	D	ECS

Ross et al. (100)	1999	Am J Surg Pathol	111	x̄ 27	**0,002**	ICM	P	Disease recurrence
Di Silverio et al. (90)	1996	Europ Urol	85	x̄ 35	**0,05**	FCM	P	
Hawkins et al. (69)	1995	Urology	894	x̄ 100	**< 0,05**	FCM	P	Partially HT
Ross et al. (51)	1994	Mod Pathol	56	x̄ 28,8	**0,0026**	ICM	P	
Tinari et al. (81)	1993	Cancer	81	84	**0,0044**	FCM	P	Stages T1 – T4
Voges et al. (71)	1993	Eur Urol	85	70	**0,001**	FCM		Time to recurrence
Montgomery et al. (72)	1990	Arch Surg	261	240	**0,001**	FCM	P	Progression & cause spec. survival
Nativ et al. (63)	1989	Mayo Clin Proc	38	94,8	**0,002**	FCM	P	GS low-grade subgroup
After TUR								
Oxford level 3b								
Nielsen et al. (241)	1993	APMIS	79	120	**0,0035**	FCM	P	Grading acc. to Shelley
After external radiation								
Oxford level 2b								
Pollack et al. (97)	2003	J Clin Oncol	149	108	**0,03**	ICM	P	Survival
Oxford level 3b								
Song et al. (240)	1992	J Urol	65	>120	**<0,0001**	ICM	P	Mayo Grade
After brachytherapy								
Oxford level 2b								
Stephensen et al. (95)	1987	Cancer Res	82	x̄ 91,8	0,0109	FCM		Pelvic lymphnode dissection, D1, N+
Oxford level 3b								
Peters-Gee et al. (170)	1992	Cancer	51	x̄ 52	**< 0,05**	ICM		
After hormonal therapy								
Oxford level 2b								

Martinez-Jabaloyas et al. (78)	2004	Actas Urol Espan	54	120	**0,009**	ICM	P	All with bone metastases
Martinez-Jabaloyas et al. (96)	2002	Urology	127	> 120	**0,031**	FCM	P	
Oxford level 3b								
Pollack et al. (97)	2003	J Clin Oncol	149	x̄ 96	**0,005**	ICM	P	After external radiation
Ahlgren et al. (85)	1997	Urology	96	176	**0,0004**	ICM	P	FNABs
Forsslund et al. (86)	1996	Cancer	334	360	**0,001**	ICM	P	FNABs
Vesalainen et al. (50)	1994	Br J Cancer	101	x̄ 156	**0,058**	FCM	P	
Di Silverio et al. (90)	1992	Eur Urol	80	x̄ 60	**< 0,05**	FCM	P	
Fordham et al. (92)	1986	Br J Surg	72	6-144	**< 0,001**	FCM	P	Ploidy + GS better GS alone
After active surveillance								
Oxford level 2b								
Adolfson et al. (76)	1990	J Urol	146	x̄ 50	**0,018**	FCM	Non-Progression	FABs. Therapy if progressed
After TUR								
Oxford level 2b								
Borre et al. (73)	1998	Prostate	**120**	x̄ 180	**0,024**	FCM	P	96 WHO low grades only

Table 7:

Addition of indenpendent 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	Flow- / Image Cytometry	Comment
After RPE							
Oxford level 2b							
Ross et al. (49)	1999	Am J Surg Pathol	111	x̄ 27	**0,002**	ICM	
Ross et al. (51)	1994	Cancer	89	x̄ 31,2	**< 0,001**	ICM	3 x more frequent
Zincke et al. (61)	1992	Cancer	370	x̄ 60	< 0,0001	FCM	Plus hormonal treatment
Montgomery et al. (72)	1990	Arch Surg	283	x̄ 112,8	< 0,001	FCM	Stage B
Winkler et al. (65)	1988	Mayo Clin and Foundation	91	>60	< 0,0001	FCM	Stage D1
After hormonal therapy							
Oxford level 2b							
Eskelinen et al. (102)	1991	Eur Urol	35	x̄ 187	0,028	FCM	T1/2,
After Brachytherapy							
Oxford level 2b							
Keyes et al. (98)	2013	In J Rad Oncol Biol Phys	94	x̄ 90	0,011	ICM	PSA recurrence

Table 8:

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

Athors	Year	Journal	Number of patients	Months of folow-up	Significance p	Flow- / Image Cytomery	Lymphnodes / Bone	Remarks
After RPE								
Oxford level 2b								
Ross et al. (52)	1994	Cancer	89	x̄ 31,2	**0,006**	ICM		
Ross et al. (103)	1993	Cancer	100	At primary diagnosis	0,0001	ICM	L & B	71 after laparatomy
Winkler et al. (65)	1988	Mayo Clin Rep	91	x̄ 90	<0,0001	FCM	B	D1
Oxford level 3b								
Ross et al. (52)	1994	Mod Pathol	56	x̄ 28,8	**0,0026**	ICM	L, B	
Oxford level 4								
Tucci et al. (153)	1994	Brazilian J Med Biol Res	28	x̄ 50	0,03	ICM	B	
After hormonal therapy								
Oxford level 3b								
Tribukait (242)	1993	Eur Urol	309	176	**< 0,0001**	FCM		
Eskelinen et al. (102)	1991	Eur Urol	91	x̄ 187	**0.0601**	FCM	Ln	

Table 9:

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

Authors	Year	Publica-tions reviewed	Syste-matic	Flow- / Image Cytometry	Metho-dological aspects	Prognostic significance	Comparison with other markers
Buhmeida et al. (109)	2006	14	No	FCM & ICM	Yes	„Predicts P significantly in organ confined disease“	Yes N = 7
Montironi et al. (108)	2006	2	No	FCM	No	Not done	No
Epstein et al. (12)	2005	18	No	FCM & ICM	Yes	„Ploidy looks promising following RPE“	Yes N = 16
Ross et al. (110)	2003	8	No	FCM & ICM	No	DNA-ploidy = CAP category II	Yes N = 28
Chakravanti and Zhai (111)	2003	8	No	FCM & ICM	No	Predicts P independtently	Yes N = 29

Mazzuchetti et al. (112)	2002	8	No	FCM & ICM	No	„Provides important prognostic information“	Yes N = 1
Miller et al. (113)	2001	6	No	FCM & ICM	No	„Questionable independent variable“	Yes N = 3
Bostwick et al. (114)	2000	5	No	FCM & ICM	No	DNA-ploidy = CAP category II	Yes N =6
Sakr and Grignon (115)	1997	16	No	FCM & ICM	No	„Good potential as prognostic marker“	Yes N = 3
Mikuz (116)	1997	4	No	FCM & ICM	No	„Difficult to understand why these well documented data have not yet gained access to treatament protocols“.	No
Schröder et al. (117)	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 studied in clinical trials, particularly in patients with localized cancer“.	No
Shankey et al. (31)	1993	?	No	FCM	Yed	„Any sample shown to contain representative tumor can provide meaningful information“.	
Lieber (118)	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. (119)	1992	8	No	FCM	No	„FCM has much to tell us about the natural history and biologic behaviour of prostate cancer“.	No
Böcking (120)	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

Table 10:
Reviews dealing with DNA-cytometry in prostate cancer