Clinical utility of kallikrein-related peptidases (KLK) in urogenital malignancies

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Summary

Kallikrein-related peptidases (KLK), which represent a major tissue-associated proteolytic system, stand for a rich source of biomarkers that may allow molecular classification, early diagnosis and prognosis of human malignancies as well as prediction of response or failure to cancer-directed drugs. International research points to an important role of certain KLKs in female and male urogenital tract malignancies, in addition to cancers of the lung, brain, skin, head and neck, and the gastrointestinal tract. Regarding the female/male urogenital tract, remarkably, all of the KLKs are expressed in the normal prostate, testis, and kidney whereas the uterus, the ovary, and the urinary bladder are expressing a limited number of KLKs only. Most of the information regarding KLK expression in tumour-affected organs is available for ovarian cancer; all of the 12 KLKs tested so far were found to be elevated in the malignant state, depicting them as valuable biomarkers to distinguish between the normal and the cancerous phenotype. In contrast, for kidney cancer, a series of KLKs was found to be downregulated, while other KLKs were not expressed. Evidently, depending on the type of cancer or cancer stage, individual KLKs may show characteristics of a Janus-faced behaviour, by either expanding or inhibiting cancer progression and metastasis.

Keywords

Cancer, proteases, endometrium, ovary, prostate

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Introduction

In the United States of America (USA), for the year 2012, 848,170 new cancer cases were predicted for the male population, and 790,740 new cases for women. Of that, 251,900 new cancer cases (29.7%) would affect the male reproductive system compared to 88,750 (11.2%) cases affecting the female reproductive system (1, 2). For the urinary system, an estimated 97,610 new cases (11.5%) were estimated for men and 43,530 new cases (5.5%) for females. All of these cancers can invade nearby organs or metastasise through the bloodstream or lymphatic system to other, distant parts of the body.

Gynaecological cancers include the more common cancers of the corpus uteri (endometrium), cervix uteri, and the ovaries, in addition to the rare cancers of the vagina, vulva, and the fallopian tubes. In the western world, the incidence of gynaecological malignancies is highest for endometrial cancer, followed by cervical and ovarian cancer, while the mortality rate is quite opposite: highest for ovarian cancer, followed by cervical and endometrial cancer (3). Staging of all of the gynaecological malignancies is performed according to the guidelines of the International Federation of Gynaecology and Obstetrics (FIGO) (4). With an 349,510 new cases estimated, cancers of the male urogenital tract encompassing those of the prostate, testis, penis, plus kidney and bladder, accounted for over 41.2% of all new cases estimated. Within the group of genital cancers, prostate cancer is the most frequent cancer in men (estimated 241,740 new cases, 24.5%) and testicular cancer (estimated 8,590 new cases, 1.1%) most common in young men (1, 2).

Current research aims at understanding the biology underlying these disease processes, especially understanding the complex molecular interactions within a tumour and molecular actions of drugs. To identify targets for new and novel therapies remains important to improve our knowledge of the pathobiology of these cancer diseases and associated chemoresistance. Cancer biomarkers such as the kallikrein-related peptidase family members (KLK) may help to identify patients at risk and to see how well a body afflicted with cancer responds to cancer therapy and may even help to decide how aggressive the cancer treatment should be (5, 6).

Expression of KLKs (kallikrein-related peptidases) in the urogenital system

KLK1–15 comprise 15 highly conserved serine proteases with similar structural characteristics and a wide spectrum of functional properties (7). In the female reproductive system, the uterus, endometrium, cervix uteri, and the ovarian and fallopian tube tissues show a variable pattern of expression among the 15 KLK family members (8). During the menstrual cycle, the innermost glandular layer of the uterus, the endometrium, grows to a thick glandular tissue layer, and shows expression of most of the KLKs, both in the proliferative and the secretory phase. Variable expression of KLKs is observed in the mucin-secreting epithelium of the endocervix and the tubular cervical glands as well (9, 10). Different from these tissues, a few KLKs are highly expressed within the ovary and/or the fallopian tube, others are generally absent or only weakly expressed (11-14) (►Tables 1-3).

In the male reproductive system, prostatic tissues show high expression of selected KLKs (8, 15) (►Table 4 and ►Table 5). Assessment of the topographical distribution of several of the KLK antigens by immunohistochemistry showed mostly cytoplasmic staining in the luminal secretory cells of the prostatic epithelium, in basal cells, and in foci of basal cell hyperplasia. As an exception, KLK4 appears to be located to the nucleus as well (16, 17). The epithelium of the rete testis, ejaculatory ducts, seminal vesicles, ductus deferens, epididymis efferent ductules, penile urethra and Littre's and Cowper's glands express KLKs as well; the spermatic epithelium in the testis shows variable expression of KLKs with spermatogonia being the predominant KLK expressing cell population (11-14, 17).

The urinary system includes the kidneys, bladder, and ureter. In the normal kidney, there is relatively low mRNA expression of several of the KLKs but high expression of others (►Table 6) (8, 12, 13). In the normal urinary bladder, so far, expression patterns of only a few of the KLKs have been investigated (8). Surprisingly, urine contains only few KLKs, e.g. KLK1, 3, 4, 9, and 12, present at low levels (8).

Deregulated expression, secretion, and function of various KLK family members have been reported for solid malignant tumours of the urogenital tract, including kidney cancer, cervical cancer and hormone-dependent cancers of the endometrium, ovary, prostate, and the testis. In these cancers, KLKs may serve as diagnostic, prognostic, or therapy-response cancer biomarkers and may even help to distinguish between various types of cancer when morphology is similar (18). Still, there is not a uniform pattern concerning mRNA or protein expression of the various KLKs under malignant conditions. KLKs are downregulated in kidney cancer but upregulated in ovarian cancer, compared to their non-malignant cellular counterparts (15, 17, 18); while some of the KLKs are downregulated in prostate cancers, others are upregulated (15, 17, 19).

Clinical utility of KLKs in cancers of the female reproductive tract

KLKs in endometrial cancer

Endometrial cancer originates from the inner glandular layer of the uterus. The leading and early symptom of endometrial cancer is vaginal discharge or abnormal bleeding. Because of this, en-

Table 1: KLKs present in normal tissues and tumour tissues of patients afflicted with endometrial cancer.

	Normal (uterus)		Normal (endometrium)		Cancerous+		References
	mRNA	Protein	mRNA	Protein	mRNA	Protein	
KLK1	Low	Low	Expressed	Expressed	↓	ND	8, 44, 142, 143
KLK2	Absent	Absent	Expressed	ND	ND	ND	8, 144
KLK3	Low	Absent	Expressed	Expressed	ND	ND	8, 144, 146
KLK4	Absent	Low	ND	Expressed	ND	↑	8, 25, 44
KLK5	Absent	Absent	ND	Expressed	ND	ND	8, 9, 16, 27
KLK6	Absent	Low	Expressed	Expressed	↑	ND	8, 9, 12, 16, 26, 27, 44, 145
KLK7	Absent	Absent	ND	Expressed	ND	ND	8, 16, 27, 44
KLK8	Absent	Absent	Expressed	Expressed	↑	↑	8, 10
KLK9	Absent	Moderate	ND	ND	ND	ND	6, 8
KLK10	Moderate	Absent	Expressed	Expressed	↑	ND	8, 13, 16, 44, 145
KLK11	Low	Low	ND	Expressed	ND	ND	8, 9, 16, 27, 44
KLK12	Low	Moderate	ND	Expressed	ND	ND	8, 9, 16, 27, 44
KLK13	Absent	Low	ND	Expressed	ND	ND	8, 9, 16, 27
KLK14	Low	Low	ND	Expressed	ND	ND	8, 16, 56
KLK15	Absent	Absent	ND	ND	ND	ND	8

↑ = Increased, ↓ = decreased compared to normal tissue. ND = Not determined, +Endometrial cancer.

Table 2: KLKs present in normal tissues and tumour tissues of patients afflicted with cervical cancer.

	Normal		Cancerous		References
	mRNA	Protein	mRNA	Protein	
KLK1	Moderate	Low	ND	ND	8
KLK2	Low	Absent	ND	ND	8
KLK3	Low	Absent	ND	ND	8
KLK4	High	Low	ND	ND	8
KLK5	High	Low	ND	ND	8, 9, 16
KLK6	High	Low	ND	ND	8, 9, 16
KLK7	High	Low	ND	↑	8, 16, 31, 32
KLK8	High	Low	ND	↑	8, 34
KLK9	High	Moderate	ND	ND	8
KLK10	High	Low	ND	ND	8, 16
KLK11	High	Moderate	ND	ND	8, 9, 16
KLK12	Low	Moderate	ND	ND	8, 9, 16
KLK13	High	Low	ND	ND	8, 9, 16
KLK14	Moderate	Low	ND	ND	8, 16
KLK15	Absent	Absent	ND	ND	8

↑ = Increased, ↓ = decreased compared to normal tissue. ND = Not determined.

dometrial cancer is often diagnosed in an early stage, giving hope for a favourable clinical course of the disease. The metabolic syndrome, comprising obesity, diabetes, and dislipidaemia, as well as infertility, polycystic ovarian syndrome, Lynch syndrome, and early menarche and late menopause are known risk factors for endometrial cancer (20, 21). Therapy of choice of endometrial cancer is hysterectomy with bilateral salpingo-oophorectomy, often associated with pelvic and paraaortal lymphadenectomy and/or followed by adjuvant radiotherapy. Chemotherapy or endocrine therapy is primarily administered in advanced stages of endometrial cancer (22). Currently, no effective serological or tissue biomarkers exist to classify patients at risk. The serum biomarker CA125 (a tumour cell surface mucin encoded by the *MUC16* gene) can be used in single cases to monitor effect of therapy, but one has to keep in mind that only 25% of the endometrium cancer patients are positive for CA125 (23).

Published data are available regarding KLK mRNA and/or protein expression in tissue extracts of normal uterus/endometrium and its malignant counterparts (8) (▶Table 1). Immunoenzymometric testing of tissue extracts of the uterus revealed that eight KLK proteins were expressed (KLK1, 4, 6, 9, and 11-14), seven were not. At the mRNA level, six of the KLKs were detected (KLK1, 3, 10-12, and 14). Limited data are available for KLK expression in the normal endometrium, at the mRNA level and at the protein level, as assessed by immunohistochemistry. Six KLK mRNAs (KLK1-3, 6, 8, and 10) were found to be expressed, for the other nine KLKs no mRNA expression data have been reported. Assessment by immunohistochemical staining demonstrated protein expression of 12 KLKs (KLK1, 3-8, and 10-14), no data are available regarding protein expression in the normal endometrium of the other three KLKs (16). Not much of published information is available regarding the mRNA/protein expression patterns of KLKs in endometrial carcinomas. At the mRNA level, KLK1 was found to be downregulated, KLK6, 8, and 10 are upregulated. KLK4 and 8 proteins are upregulated; no data were presented in the scientific literature regarding protein expression of the other 13 KLKs.

KLK4, which is overexpressed in endometrial cancer compared to normal and hyperplastic endometrium as assessed by immunohistochemistry, is upregulated by the steroid hormones oestrogen and progesterone (24, 25). In contrast, KLK6 has emerged as a marker describing a more aggressive type of this cancer disease: in type-II serous papillary endometrial cancer, KLK6 gene expression is significantly elevated compared to normal, benign, and type-I endometrioid carcinoma; KLK6 and 10 proteins are significantly elevated in the blood of patients presenting with serous-papillary endometrial carcinoma (26, 27).

KLKs in cervical cancer

Malignant tumours of the cervix uteri are one of the leading causes of death of young women worldwide, though due to screening programs and successful therapy of pre-malignant lesions and early stages of the disease, cervical cancer has become a rare disease in the industrialised world. Cervical cancer is known to develop stepwise from infection with the human papilloma virus (HPV) and subsequent inefficient immune response to eliminate the virus, followed by cervical dysplasia (CIN I-III), eventually leading into an invasive type of cervical carcinoma (28). One of the most important factors to predict the clinical outcome of the disease is clinical stage at the time of diagnosis. Screening by regular gynaecological examination, together with the result of the PAP smear test (the microscopical examination of cervical cells stained according to Papanicolaou) can effectively diagnose premalignant lesions or early cancer stages and thus reduce incidence and mortality of cervical cancer. Management of cervical cancer is stage-dependent: the early invasive cervical cancer is a domain of the surgeon, performing a total radical hysterectomy including dissection of the parametries and pelvic lymph nodes, and resection of the vaginal cuff. In the advanced stages of cervical cancer, primary radio-chemotherapy is indicated (29).

To date, tumour markers play a minor role in the management of cervical cancer. For the squamous cell carcinoma type, efficacy of therapy can be monitored by determination of blood level squamous cell carcinoma antigen, or, for the adenocarcinoma-type, the carcinoembryonic antigen (CEA) and CA125. Admittedly, however, for any stage of cervical cancer, no effective prognostic or predictive cancer biomarkers have been established yet (30).

Although it is known that all of the KLKs are expressed in the normal cervix, except KLK15, either at the mRNA or the protein level (8, 16) (▶Table 2), no mRNA KLK expression data are available and only for KLK7 and 8 protein data are published with re-

gard to their expression status in the malignant state. For instance, KLK7 is highly expressed in tumour tissue (31) and KLK7 content increases with the severity of cervical lesions, i.e. cervicitis, low-grade cervical intraepithelial neoplasia, high-grade cervical intraepithelial neoplasia, squamous cervical carcinomas, and even cervical adenocarcinomas (32). Thus, KLK7 could be a useful marker additional to the PAP smear for screening of cervical precursor lesions (32). Interestingly, the endogenous antileukoprotease inhibitor (ALP/SLPI) of KLK7 is significantly reduced in cervical adenocarcinomas when compared with normal endocervical glands, pointing to a regulatory role of KLK7 and ALP/SLPI in the endometrium (33). As shown for a few tissue specimens only, the KLK8 protein is also expressed in cervical carcinomas although these data have not been validated yet (34).

KLKs in ovarian cancer

The general term "ovarian cancer" encompasses those tumours of the ovary originating from the epithelial surface of this organ and accounts for more than 80% of all solid malignant ovarian tumours. Ovarian carcinomas are categorised according to their histology or molecular subtype. The most common histological epithelial subtype is that of serous cystadenocarcinoma, followed by endometrioid and mucinous carcinoma. Epithelial ovarian cancers are classified molecularly into type I and type II (35), with type I ovarian cancers including low-grade serous-papillary, endometrioid, and borderline tumours of low malignant potential. Type II ovarian cancers are more frequent (75%) and encompass high-grade serous carcinomas, undifferentiated carcinomas, and carcinosarcomas.

Prognosis of patients afflicted with ovarian cancer is poor owing to late diagnosis and often inefficient primary debulking surgery, but also because of rapidly developing chemoresistance. Potentially, early detection of ovarian cancer could decrease mortality, but unfortunately, there is no effective screening procedure: neither vaginal ultrasonography nor analysis of serum cancer biomarker CA125 nor any other biomarker analyses at the protein or gene level are specific and sensitive enough for that objective. Thus, ovarian cancer diagnosis is performed by histological inspection of the biopsy. As a result, a number of patients will have laparotomy though only suffering from a previously unknown benign ovarian tumour operable via laparoscopy. In the case of an adnexal mass of unknown dignity, presurgical assessment of CA125 in serum as well as the cancer biomarker tests OVA1 or ROMA can be applied to support a clinician's decision to perform a biopsy or choose the best surgical approach (37).

Yet, about 20% of ovarian cancer patients do not express the CA125 antigen. Similarly, with many false-positive cases for OVA1 and ROMA, it appears to be useful only for preventing a malignant mass to be falsely classified as benign (36). On the other hand, serum biomarker CA125 is established as a useful cancer biomarker to monitor response of CA125-positive ovarian cancer patients to cancer therapy.

For ovarian cancer, the major traditional prognostic factor is FIGO stage at time of diagnosis. For instance, 5-year-survival rate of early FIGO stage I patients is >90% while survival of patients with late FIGO stages III or IV is <25%. Another important traditional prognostic factor is size of residual tumour mass after cytoreductive surgery (38). Regrettably, owing to the lack of suitable biomarkers, at present, ovarian cancer management is not considering any prognostic or therapy response predicting factors, concerning the course of the disease or a patient's risk to develop disease recurrences (36).

Since KLKs are supposed to contribute to ovarian cancer progression and metastasis, recently, broad scientific interest has focused on these novel cancer biomarkers aiming at exploring their clinical validity (15, 19, 39). For several of the KLKs, both mRNA and protein levels have been reported to be increased in ovarian cancer tissue, compared to levels found in physiological situations (▶Table 3).

By enzymometric determination of bulk-extracted normal ovarian tissues, seven KLKs were found to be expressed in the normal ovary (KLK1, 6-8, 10, 11, and 14) (8); immunohistochemical staining revealed positivity for KLK5, 12, and 13 as well (16). At the mRNA level, nine KLKs are expressed (KLK1, 6-11, 13, and 14) (8). Ten KLK mRNAs (KLK3-8, 10, 11, 13, and 15) were found to be elevated in ovarian cancer tissue compared to normal counterparts, KLK14 is downregulated (40-52). When assessing KLKs at the protein level by immunohistochemistry or ELISA, 12 KLKs (KLK3-11, and 13-15) were found to be upregulated in ovarian cancer compared to normal ovarian tissues or other non-malignant gynaecological diseases (38, 44, 53-55).

Five KLK family members (KLK4-7, and 15) are linked to poor prognosis of ovarian cancer patients, two (KLK9 and 14) are associated with a favourable course of the cancer disease. For some KLKs (KLK8, 10, 11, and 13), the clinical relevance is not clear yet, since, depending on the way of detection (mRNA or protein) and FIGO stage (early or advancend), these KLKs may be a factor to either predict a poor or a good course of the cancer disease (56). The prognostic or predictive value of KLK1, 2, and 12 has not been determined yet; KLK3 expression was found not to be correlated with clinical outcome; still, presence of the *KLK3* rs11084033 allele correlated with poor survival as determined by the Sequenom iplex mass array technology (57).

More specifically, *KLK4* mRNA expression in ovarian tumour tissue is associated with untimely disease recurrence and death (58). This feature parallels that of KLK5 expression in ovarian tumour tissue where elevated *KLK5* mRNA and KLK5 protein is correlated with shorter disease-free and overall survival (59, 60). Analysis of KLK6 protein in tumour cytosols using ELISA indicates that KLK6-positive tumours are more likely associated with advanced disease, serous histology, and suboptimal debulking. KLK6 impacts on overall and progression-free survival, especially in ovarian cancer subgroups with a good prognosis at first sight, e.g. with low-grade tumours and optimally debulked patients (61). Further, stromal-cell associated overexpression of KLK6 protein as assessed by immunohistochemistry is associated with shorter overall and progression-free survival as well (62). High concentrations of *KLK7* mRNA and KLK7 protein in ovarian tumour tissue were reported to be associated with advanced stage and larger

	Normal		Cancerous		References
	mRNA	Protein	mRNA	Protein	
KLK1	Moderate	Moderate	ND	ND	8, 130
KLK2	Absent	Absent	ND	ND	8, 130
KLK3	Absent	Absent	↑	↑	8, 50, 57, 130, 147, 148
KLK4	Absent	Absent	↑	↑	8, 43, 58, 84, 130, 149
KLK5	Absent	Present	↑	↑	8, 16, 44, 48, 55, 59, 60, 130, 149–151
KLK6	High	Moderate	↑	↑	8, 16, 42, 44–46, 50, 55, 61, 62, 69, 75, 130, 149, 150, 152–159
KLK7	High	Moderate	↑	↑	8, 16, 42, 44, 45, 47, 48, 53, 63, 85, 130, 149, 150, 160
KLK8	High	Low	↑	↑	8, 42, 44, 45, 50, 65–69, 130, 149, 150
KLK9	Low	Absent	ND	↑	8, 64, 130
KLK10	High	Moderate	↑	↑	8, 16, 38, 42, 44, 45, 49, 50, 55, 70, 86, 130, 149, 150, 155, 158
KLK11	Moderate	Moderate	↑	↑	8, 16, 44, 45, 55, 71, 72, 74, 130
KLK12	Absent	Present	ND	ND	8, 16, 130
KLK13	Low	Present	↑	↑	8, 16, 38, 55, 73, 75, 130
KLK14	Moderate	Low	↓	↑	8, 11, 16, 44, 51, 123, 130, 161
KLK15	Absent	Absent	↑	↑	8, 52, 130, 162

↑ = Increased, ↓ = decreased compared to normal tissue. ND = Not determined.

Table 3: KLKs present in normal tissues and tumour tissues of patients afflicted with ovarian cancer.

residual tumour mass, or lower nuclear grade disease and those who have been optimally debulked, as well as shorter overall and progression-free survival (53, 63). Increase in *KLK15* mRNA expression in ovarian tumour tissue is also associated with shorter survival (52). Different from *KLK4-7* and *15* expression, *KLK9* and *14* mRNA are considered factors indicating favourable clinical outcome of ovarian cancer patients (51, 64).

Conflicting results exist concerning the clinical utility of four of the KLKs (KLK8, 10, 11, and 13). For instance, expression of KLK8 protein (65, 66) and *KLK8* mRNA (67, 68) were determined to be elevated in tumour cytosols of ovarian cancer patients, and expression correlated to a favourable outcome. Contrary to these results, the immunohistochemical AQUA-study by Kountourakis classified KLK8 protein as a marker of poor clinical outcome although results of this image analysis-based technology deserve validation (69). For KLK10, Luo et al. reported that elevated KLK10 protein in ovarian cancer tumour tissue is associated with advanced stage, suboptimal debulking, larger residual tumour mass, serous histology, and shorter progression-free and overall survival (70). These data could not be confirmed by Dorn et al. who reported that low KLK10 protein expression is associated with poor survival (38). In another study, KLK11 and 13 protein levels expressed in ovarian cancer tissues were found to be correlated with early stage disease and favourable overall survival (71-73). This is different from the findings of Shigemasa et al. and White et al. (74, 75) who reported that increased *KLK11* or *13* mRNA expression values are associated with high-grade tumours and poor patient outcome. Evidently, the prognostic value of *KLK11* and *13* mRNA expression differs from the KLK11 and 13 protein expression values.

Since residual tumour mass after primary debulking is one of the most important issues in ovarian cancer, a score to predict surgical outcome based on the clinical factors tumour grading and ascites volume plus KLK6 and 13 was established by Dorn et al. (38). We argued that such a score could help to identify those ovarian cancer patients who could be spared the burden of surgery.

Seven KLKs (KLK5-8, 10, 11, and 14) were found to be released into the blood (serum) of early or advanced ovarian cancer patients, and are elevated in comparison to serum levels of healthy individuals, patients with benign diseases of the ovary, or cancer of other origin (11, 59, 66, 76-83). Nine of the KLKs are also enriched in peritoneal ascites of ovarian cancer patients (KLK5-13, not KLK4 or 14) (8, 11, 81, 84). Elevation of KLK5, 6, 10, and 11 proteins in serum are considered markers of poor prognosis (overall or progression-free survival) for the ovarian cancer patient (75, 78-83) while an increase in KLK8 in ascitic fluid indicates a favourable prognosis (66).

When evaluating the potential clinical application of KLK family members in conjunction with the monitoring cancer biomarker CA125, a direct correlation of CA125 and KLK8 expression released into the blood or the ascitic fluid was uncovered (66). Furthermore, El Sherbini et al. (82) presented evidence that the combination of CA125 with KLK6 and 10 renders increased specificity to predict for ovarian cancer, when compared to the clinical im-

pact of each of the biomarkers alone (78, 83). Additionally, when CA125 was determined in combination with KLK6 and 13 instead of KLK10, it was found that the combination of these three biomarkers is a more sensitive test to detect early stage ovarian cancer than by dermination of CA125 alone (40). Different from that approach, Rosen et al. determined KLK6 and 10 in sera of ovarian cancer patients lacking CA125 expression and reported that these markers may have the potential to serve as sensitive markers for early detection of ovarian cancer (37).

Apart from their prognostic value, expression of several of the KLKs in tumour tissues or release into the blood/ascites is also measured to foresee response to adjuvant cancer therapy. For instance, for a cohort of ovarian cancer patients with recurrent disease, KLK4 protein expression as assessed by immunohistochemistry was determined to be elevated in tumour tissues of the group of patients progressing under treatment with the chemotherapeutic drug taxane (paclitaxel), but not in the group of responders (85). Likewise, there is a direct correlation between tumour tissue-associated elevation of KLK7 protein and failure of the ovarian cancer patients to respond to carboplatin ± taxol chemotherapy (86). Zheng et al. (55) combined determination of KLK6, 8, and 13 with cancer stage and debulking status to distinguish between responder (complete or partial) and non-responder ovarian cancer patients treated with platinum-based chemotherapy. It was also found that KLK10 expression is a predictive marker related to resistance of ovarian cancer tissues to chemotherapy (87).

Oikonomopoulou et al. (81) determined serum protein levels of KLK5-8, 10, and 11, together with the serum markers CA125, B7-H4 (a member of the B7 family of immune costimulatory proteins), regenerating protein IV, and spondin-2 at baseline, or after the first chemotherapy cycle, to predict a patient's response to carboplatin and/or taxol-based chemotherapy. The authors reported that a range of serum biomarkers, encompassing CA125 and KLK5 and 7, would predict response to chemotherapy. All of these cancer biomarkers examined, except KLK7 and regenerating protein IV, were also powerful predictors of time-to-progression among the chemotherapy responders (81). Taken as a whole, KLKs have recently emerged as novel, promising predictive factors in ovarian cancer. However, owing to the lack of sufficiently effective alternative therapy models to treat advanced ovarian cancer patients, this knowledge is of limited clinical use.

Clinical utility of KLKs in cancers of the male reproductive tract

KLKs in prostate cancer

Prostate cancer is the second most common cancer in men worldwide (3) and the leading type of cancer in men in the USA (1, 2).

Definitive diagnosis of prostate cancer is done by biopsy. Malignant transformation of the epithelial cells lining the secretory glands of the prostate may manifest in early non-invasive lesions including high-grade prostatic intraepithelial neoplasia (HGPIN) (88). At diagnosis, tumour stage, Gleason score (89), and PSA (prostate-specific antigen, also known as kallikrein-related peptidase 3, KLK3) are the most accepted important predictors of prognosis of prostate cancer.

These parameters influence treatment strategies, which may include active surveillance; or surgery with or without a combination of radiation, steroid hormone or chemotherapeutic interventions for those cancers that are deemed aggressive. The observed biological heterogeneity in prostate cancer is reflected by the range of survival among men diagnosed with the disease, with small localized cancers having the generally high 5-year average survival of >85% in industrialised countries (3), while more aggressive cancers having a much worse prognosis.

As such, current research is focusing on identifying genomic, transcriptomic, or proteomic biomarkers that distinguish between indolent and aggressive cancers for which molecular pathways may reveal therapeutic targets. To date, molecular profiling approaches have identified several potential markers, including the frequent losses of NKX3.1 (8p21) and PTEN (10q23), gains of AR and fusion of ETS family transcription factor genes with androgen-responsive promoters, amplifications of MYC, and mutations of TP53, PTEN, AR, RB1 or APC (90-94); in addition to miRNAs as potential prognostic biomarkers (95).

Prostate cancer is relatively slow growing, thus allowing a sufficient time for cancer to be discovered before it becomes incurable, for instance by use of the non-invasive PSA screening test (96-102). Yet, today, PSA's (KLK3) effectiveness for screening and diagnosis of prostate cancer is not that obvious anymore and therefore questioned by several clinicians (100). Current controversies and major doubts regarding this matter of debate are explained and discussed in further detail by Fillon et al. (101).

In the normal prostate, all of the KLKs are expressed at the mRNA level and, except for KLK8, at the protein level as well (8, 16). In prostate cancer, elevated levels of KLK2, 4, and 12-15 mRNA and/or protein have been reported; three of those (KLK2, 14, and 15) are associated with poor prognosis. Decreased mRNA or protein levels of KLK2, 3, 5-7, 10, 11, 13, and 15 have been reported of which KLK3 and 15 are associated with poor prognosis while KLK5 and 11 expression is associated with a favourable prognosis (►Table 4) (15, 102, 103).

The regulation of KLK expression in prostate cancer has been extensively studied, with evidence showing the regulatory role of various steroid hormones (104). Indeed, KLK2 and 3 possess defined steroid hormone binding sites (41, 104) while KLK1 and 4 possess putative steroid binding elements (105); the remaining KLKs fail to possess such defined elements (41, 104). Epigenetic studies suggest a role for differential DNA-methylation in KLK regulation (106-109) influencing aberrant expression. More recently, non-coding mRNAs, such as miRNAs, were suggested to be involved in the regulation of certain KLK genes (110).

Prostate cancer screening remains controversial, because of the inability of any biomarker to be detected early enough with high specificity for a significant proportion of dangerous tumours. Yet, also in combination with PSA, several promising novel blood-based biomarkers for prostate cancer diagnosis, monitoring, or prediction of the course of the disease and response to cancer therapy are under investigation. e. g. KLK2, urokinase plasminogen ac-

	Normal		Cancerous		References
	mRNA	Protein	mRNA	Protein	
KLK1	High	High	ND	ND	8, 142, 163–169
KLK2	High	High	↑	↑↓	8, 96, 102, 163–173
KLK3	High	High	↓	↓	7, 8, 96, 100, 102, 142, 163–170, 172, 174–176
KLK4	Moderate	Low	↑	↑	8, 12, 14, 16, 62, 163–169, 177–184
KLK5	Low	Low	↓	ND	8, 16, 163–169, 185, 186
KLK6	Low	Present	ND	↓	8, 12, 14, 16, 163–165, 168, 169, 180, 181
KLK7	Moderate	Present	↓	↓	8, 16, 163–169, 187–189
KLK8	Moderate	Absent	ND	ND	8, 16, 163–165, 168, 169, 190
KLK9	Low	High	ND	ND	8, 163–169
KLK10	High	Present	↓	↓	8, 12–14, 16, 109, 163–165, 168, 169, 178, 180, 181
KLK11	High	High	↓	↓	8, 16, 163–169, 187, 191–195
KLK12	Moderate	Present	ND	↑	8, 12, 14, 16, 179–181, 196–198
KLK13	Low	Low	↑	↑↓	8, 12, 14, 16, 163–169, 178, 180, 181
KLK14	High	Low	↑	↑	8, 16, 123, 163–169, 199, 200
KLK15	High	Low	↑	↓	8, 103, 163–169

↑ = Increased, ↓ = decreased compared to normal tissue. ND = Not determined.

Table 4: KLKs present in normal tissues and tumour tissues of patients afflicted with prostate cancer.

tivator (uPA) and its receptor uPAR, transforming growth factor-beta 1 (TGF-β 1) as well as interleukin-6 (IL-6) and its receptor IL-6R (111).

KLKs in testicular cancer

Testicular cancer is the most common among Caucasian men between age 15 and 35; while relatively uncommon in Asia and Africa. More than 95% of testicular cancers are germ cell tumours. Testicular cancer is very well treatable (surgery, radiotherapy, chemotherapy) with a cure rate of ~95% if it has not spread outside the testicle, due to its extreme sensitivity to cisplatin-based chemotherapy, even when metastatic (112, 113). Indeed, if it has metastasised to organs or lymph nodes, the five-year relative survival rate is still high with ~72%.

Although two highly specific and sensitive serum markers: α-fetoprotein (AFP) and human chorionic gonadotropin (hCG) are not recommended for the diagnosis of testicular cancer in the absence of histologic confirmation, they are useful markers in the management of testicular germ cell tumours (114, 115). In the last years, novel biomarkers, e.g. glypican 3, SALL4, OCT3/4, SOX2, SOX17, OCT3/4, NANOG HMGA1, HMGA2, PATZ1, GPR30, and Aurora B were reported to discriminate between testicular cancer subgroups (116-118).

In the normal testis, all of the 15 KLKs are expressed at the mRNA level (8). Compared to other normal tissues, KLK proteins are only weakly/moderately expressed in testicular tissues; KLK15 is not expressed (8, 16) (▶Table 5). Some of the KLKs have been shown to be of clinical value as biomarkers in testicular cancer as well, such as KLK5, 10, 11, 13, and 14 (119-124). mRNA expression data of these five imply, compared with normal testicular tissue, that these KLKs are downregulated in testicular cancer (119). Among those, lower mRNA levels in late stage (II/III) versus early stage (I) carcinomas indicate that KLK5 might be a marker indicating a favourable prognosis (120). This might apply to the other four KLKs tested as well; even a possible tumour suppressive role of these KLKs in testicular cancer was discussed (121-123). In one of the studies, five splice variants of KLK13 were identified, exclusively expressed in normal testicular tissue, which are not present in the malignant counterpart (122). Full-length KLK13, on the other hand, is expressed both in the normal and the cancerous testicular tissue. To date, no study results relating to testicular cancer mRNA expression have been presented for the other ten KLKs; and at present, no results are available relating to the testicular tumour protein levels of KLK1-9 and 11-15.

KLKs in kidney cancer

Renal cell carcinoma (RCC), with an estimated incidence of 4% (64,770 of 1,638,910) and estimated 2.4% (13,570 of 577,190) cancer-related deaths for the year 2012 (1, 2) in the USA, comprises a histopathologically heterogeneous group with the majority of cancers being of the clear-cell subtype (80%); while the remaining subtypes include the papillary and chromophobe RCCs (124, 125). Along with tumour stage, most RCCs are graded according to the Fuhrman nuclear grading system (126). Surgical resection is the only known effective treatment for localised RCC. The most

Table 5: KLKs present in normal tissues and tumour tissues of patients afflicted with testicular/seminal cancer.

	Normal		Cancerous		References
	mRNA	Protein	mRNA	Protein	
KLK1	Moderate	Low	ND	ND	8, 41
KLK2	Moderate	Low	ND	ND	8
KLK3	Moderate	Moderate	ND	ND	8
KLK4	High	Low	ND	ND	8, 41
KLK5	High	Low	↓	ND	8, 16, 41, 119, 120, 163, 164
KLK6	High	Low	ND	ND	8, 16, 41, 164
KLK7	High	Low	ND	ND	8, 16, 41, 166
KLK8	High	Low	ND	ND	8, 190
KLK9	Low	Moderate	ND	ND	8, 41
KLK10	High	Low	↓	Expressed	8, 16, 41, 119, 121, 163
KLK11	High	Low	↓	ND	8, 16, 41, 163
KLK12	Low	Present	ND	ND	8, 16, 41
KLK13	Moderate	Low	↓	ND	8, 16, 41, 119, 122, 164
KLK14	High	Present	↓	ND	8, 16, 41, 119, 123
KLK15	Moderate	Absent	ND	ND	8, 41, 163

↑ = Increased, ↓ = decreased compared to normal tissue. ND = Not determined.

Table 6: KLKs present in normal tissues and tumour tissues of patients afflicted with kidney cancer.

	Normal		Cancerous		References
	mRNA	Protein	mRNA	Protein	
KLK1	High	Moderate	↓	↓	8, 16, 18, 41, 129, 163, 164, 201, 202
KLK2	Low	Low	Absent	ND	8, 129, 202
KLK3	Low	Mod	↓	ND	8, 129, 202
KLK4	Low	Low	Absent	ND	8, 129, 164
KLK5	Low	Mod	↓	↓	8, 16, 129, 134, 164
KLK6	High	Moderate/High	↓	↓	8, 12, 16, 18, 41, 129, 134, 152, 163, 164
KLK7	High	Low	↓	↓	8, 16, 18, 41, 129, 164, 166
KLK8	Moderate	Low	Absent	ND	8, 129, 190, 203
KLK9	Low	Mod	↓	ND	8, 129
KLK10	Low	High	Absent	↓	8, 13, 16, 129, 134, 164
KLK11	Low	Low	Absent	↓	8, 16, 129, 134, 164
KLK12	Low	Low	Absent	ND	8, 16, 129, 164
KLK13	Low	Low	↓	ND	8, 16, 129
KLK14	Moderate	Low	↓	ND	8, 16, 41, 129
KLK15	Low	Low	Absent	↓	8, 18, 41, 129

↑ = Increased, ↓ = decreased compared to normal tissue. ND = Not determined.

common molecular change in RCC is the loss of the VHL (von-Hippel-Lindau) gene function by deletion (127), epigenetic silencing (128), or protein regulation mediated by miRNAs (129). These findings have enabled the use of therapeutic agents that affect pathways including those of the VEGF pathway and TORC1 complex. Recent genomic and transcriptomic profiling (130) have identified a number of recurrent copy-number changes including the gains/amplifications of genomic loci harbouring putative on-

Table 7: KLKs present in normal tissues and tumour tissues of patients afflicted with urinary bladder cancer.

	Normal[a]		Cancerous		References
	mRNA	Protein	mRNA	Protein	
KLK1	ND	Low	ND	ND	8
KLK2	ND	Absent	ND	ND	8
KLK3	ND	Absent	ND	Absent	8, 139, 140–142
KLK4	ND	Absent	ND	ND	8
KLK5	ND	Absent	↑	↑	8, 16, 138
KLK6	ND	Absent	↑	↑	8, 13, 16, 138
KLK7	ND	Low	ND	ND	8
KLK8	ND	Absent	↑	ND	8, 138
KLK9	ND	Moderate	↑	ND	8, 138
KLK10	ND	Absent	ND	↑	8, 12, 13, 16
KLK11	ND	Absent	ND	↑	8, 16
KLK12	ND	Absent	ND	ND	8
KLK13	ND	Low	ND	ND	8
KLK14	ND	Moderate	ND	ND	8
KLK15	ND	Absent	ND	ND	8

↑ = Increased, ↓ = decreased compared to normal tissue. ND = Not determined.

cogenes such as MCM2, EGFR, and SEC61G; and losses of genomic regions harboring putative tumour suppressor genes including KIF1B, ARID1A, NHEJ1, KU80, and IGFBP5, among others. Molecular profiling approaches have a great potential for improving RCC patient management (131). Moreover, recent transcriptomic profiling has identified two putative molecular subtypes of clear cell RCC with implications for outcome (132), and enabled accurate molecular classification of RCC subtypes (133).

The normal kidney expresses all of the 15 KLKs, both at the mRNA and protein level (8, 16) (►Table 6). Petraki et al. (16) showed by immunohistochemistry that the eight KLKs they investigated (KLK5-7, and 10-14) are expressed by the epithelium of the urinary tubuli of the kidney while glomeruli are negative. In RCC, there is a consistent decrease (KLK1, 3, 5-7, 9-11, and 13-15) either at the mRNA or protein level or absence (KLK2, 4, 8, 10-12, and 15) of the KLKs at the mRNA level (16, 18, 129, 134). Additionally, studies by Gabril et al. (18) revealed differential immunostaining pattern of KLKs in RCC when compared to normal kidney tissue. KLKs were also found to be differentially expressed in the different RCC subtypes. There was an overall decrease in KLK1 expression in clear cell RCC compared to adjacent normal kidney; although relatively stronger KLK1 immunoreactivity was seen in high-grade compared with low-grade tumours. These findings were supported by those of White et al. (129), showing that the decreased expression of KLK1 and 3 was significantly associated with the clear cell subtype.

KLK1 showed weak expression in papillary RCC and negative to weak focal expression in chromophobe RCC and oncocytomas. In clear cell RCC, there was decreased KLK6 expression in higher grade compared with lower grade cancers. Papillary RCC showed strong diffuse KLK6 cytoplasmic protein expression with focal apical accentuation. In contrast, oncocytoma and chromphobe RCC showed no expression in tumour cells with only KLK6 expression in the blood vessels of the stroma. For KLK7, there was decreased protein expression observed in high-grade compared to low-grade-tumours. Oncocytomas showed diffuse, strong granular cytoplasmic expression of KLK7 protein while chromophobe RCC showed focal weak homogeneous cytoplasmic staining. Papillary RCC showed strong, apical cytoplasmic KLK7 protein staining. For KLK15 protein, clear cell RCCs showed cytoplasmic staining, weak to negative expression in papillary RCCs, and no expression in chromophobe RCC, urothelial carcinomas, or oncocytomas. Though still in its early stages, these findings indicate a good potential for the use of KLKs as adjuvant tissue markers to distinguish RCC subtypes.

Most of the potential clinical utility of KLKs in RCC is related to their prognostic value. KLK5, 6, 10, and 11 were assessed for their potential to predict the course of the RCC disease (134). It was only that KLK6 was of prognostic value to predict disease-specific survival; KLK5, 10, and 11 were of no effect (134).

KLK expression may be regulated transcriptionally and/or post-transcriptionally (102, 105, 106, 135). White et al. (129) demonstrated that the differential mRNA expression of KLKs in RCC may be mediated by the aberrant expression of miRNAs. Specifically, luciferase assays demonstrated the ability of hsa-let-7f to target the 3'untransled region (UTR) of KLK10 in the RCC cell line ACHN, while transient transfection of miR-224 to HEK-293 cells resulted in the decrease of *KLK1* mRNA expression.

KLKs in urinary bladder cancer

Urinary bladder carcinomas rank ninth in overall cancer incidence worldwide and are found more commonly among men than women. The vast majority of bladder cancers are of the urothelial type, superficial, and are typically treated by transurethral resection or intravesical immunotherapy and chemotherapy (136, 137). For these patients, the 5-year survival rate is nearly 90%. However, approximately 50-70% of tumours recur with some evolving into a more invasive phenotype. Patients who present with the infiltrative type have only a 60% 5-year survival probability (136, 137).

Approximately 10-15% of the non-invasive lesions progress into the muscle invasive-disease. Molecular genetics and cytogenetics have identified alterations of chromosome 9 to the normal urothelium as the earliest change prior to the divergence into the two different pathways. Disruptions of the PI3K-AKT axis and alterations of HRAS are generally associated with the non-invasive subtype; whereas alterations in genes affecting cell cycle and apoptosis include *TP53*, *CDKN2A*, *CCND1*, *CDKN1B*, or *RB*.

To date there is relatively little known about the expression of KLKs in the normal urinary bladder or in bladder cancer and their

potential use for clinical management of that malignancy. No information is available in the scientific literature concerning the mRNA expression levels in the normal bladder of any of the KLKs. Regarding the KLK protein expression in the urinary bladder, KLK1, 7, 9, 13, and 14 are expressed, the other 10 KLKs are absent (▶Table 7). For most of the KLKs, their expression levels in urinary bladder carcinoma were not investigated, except for *KLK5, 6, 8,* and *9 KLK mRNA* and KLK5, 6, 10, and 11 protein, which are all increased compared to the normal tissue counterpart (16, 18, 138). Other studies have focused on the putative clinical utility of KLK3 in bladder cancer. These studies have shown and confirmed the lack of protein expression of KLK3 in most of the bladder cancers investigated (139-141).

Perspective

This review summarises the clinical importance of various KLKs in several types of female and male urogenital malignancies, to

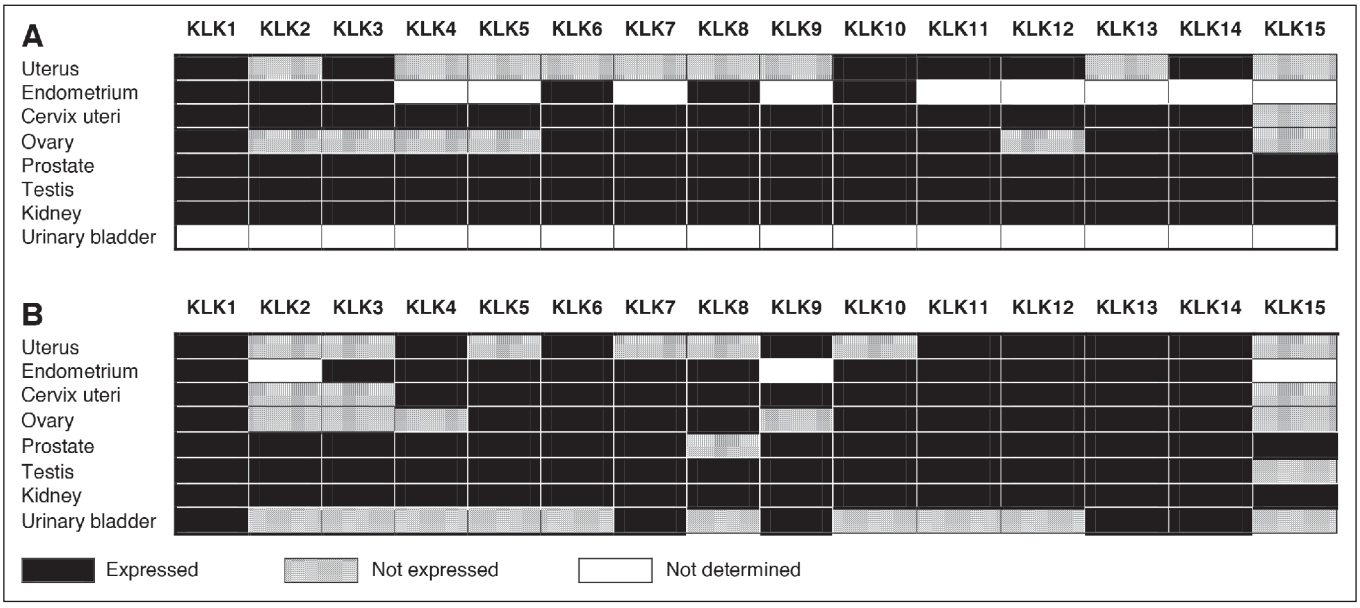

Figure 1: Comparative mRNA (A) and protein (B) expression in normal tissues of the urogenital tract.

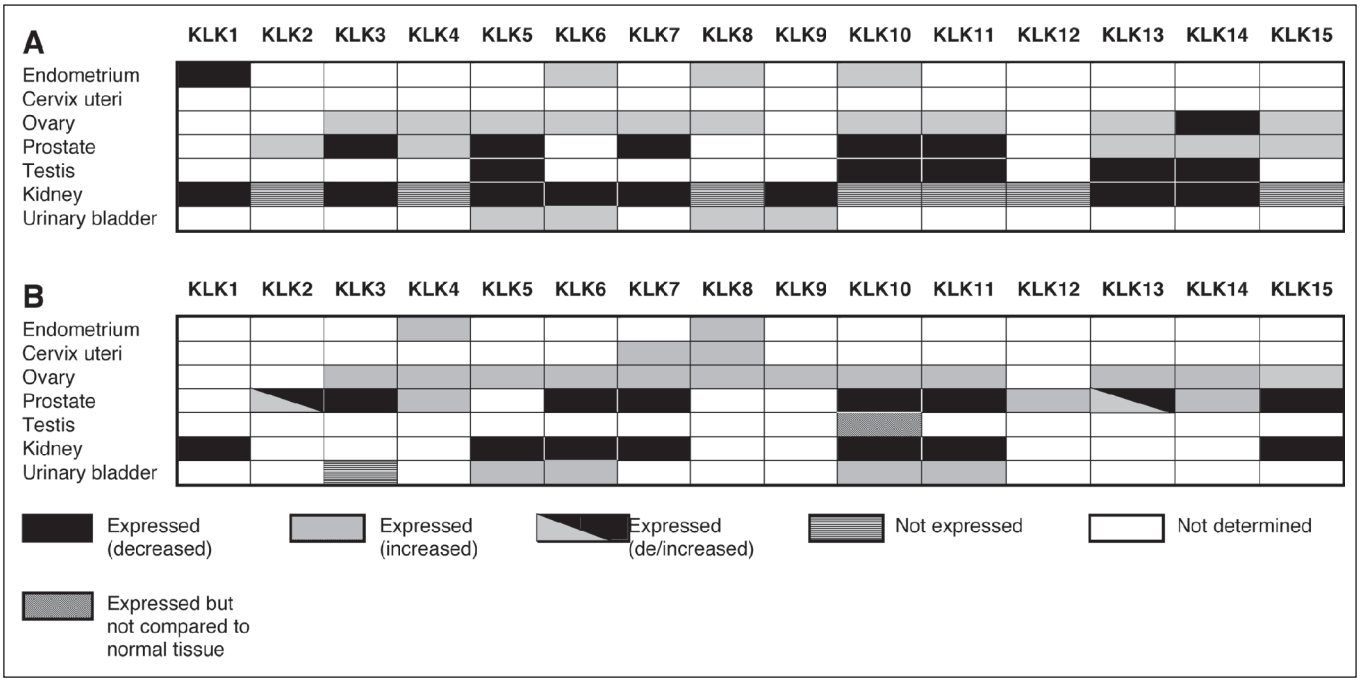

Figure 2: Comparative mRNA (A) and protein (B) expression in cancerous tissues of the urogenital tract.

give an overview and interpretation of the clinical impact of certain KLKs in these types of cancers. Although KLKs are primarily known for their biomarker value in prostate, ovarian, breast, and gastrointestinal cancers, regarding prediction of the course of the disease and response to cancer therapy, more recent data point to an important role of certain KLKs in other malignancies as well, such as those of the lung, brain, head and neck, the kidney, the endometrium, cervix, testis, and the urinary bladder (7, 15, 204).

KLKs, which represent a major tissue-associated proteolytic system, stand for a rich source of biomarkers that allow molecular classification, early diagnosis and prognosis of human malignancies as well as prediction of response or failure to cancer-directed drugs (7, 15, 204, 205). Without a doubt, these data point to the important role of various KLKs for improving individualized cancer therapy and thus clinical management of the cancer patients, especially of those patients who are likely to respond, while directing those who would not benefit towards alternative therapeutic options.

Regarding KLK expression in female and male urogenital organs and their tumour--affected counterparts, a large number of the KLKs are expressed in the normal, unaffected organs, at the mRNA and/or protein level (►Figure 1), with KLK1 and 14 expressed in all of the organs listed. Remarkably, all of the KLKs are expressed in the normal prostate, testis, and kidney whereas the uterus, the ovary, and the urinary bladder are expressing a limited number of KLKs only.

Regarding the KLK expression levels present in malignant urogenital tumours, remarkably, expression data for KLK1 (also known as tissue kallikrein) have been published only for endometrium and kidney cancer. Most of the information regarding KLK expression in tumour-affected organs is available for ovarian cancer; all of the 12 KLKs tested were found to be elevated in the malignant state, depicting them as valuable biomarkers to distinguish between the normal and the cancerous phenotype. Different from that, in kidney cancer, a series of KLKs were found to be downregulated, other KLKs were not expressed (►Figure 2).

Evidently, depending on the type of cancer or cancer stage, individual KLKs may show characteristics of a Janus-faced behaviour, by either expanding or inhibiting cancer progression and metastasis. Therefore, in order to interpret the clinical impact of different KLKs on the broad range of malignancies, the exact mechanisms of action of KLKs in distinct tumour entities and their microenvironments will need to be further elucidated.

KLKs often work in cascades by activating each other or function in signaling pathways involving kinases, phosphatases, and other proteases such as the urokinase-type plasminogen activator (uPA) (206). At first glance, the KLK proteases are characterised by high sequence similarities, which may pose many challenges impacting the development of highly specific inhibitors. Yet, KLKs do show significant differences in their substrate specificities, which have facilitated development of targeted KLK inhibitors (207). Some of the novel KLK-directed therapeutics have been tested in experimental animal models; two, affecting KLK3 (PSA) are already in phase II/III clinical trials (205).

Conflict of interest
None declared.

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