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Predictions for the future of kallikrein-related peptidases in molecular diagnostics

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EXPERT REVIEWS

Predictions for the future of kallikrein-related peptidases in molecular diagnostics

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Kallikrein-related peptidases (KLKs) form a cancer-related ensemble of serine proteases. This multigene family hosts the most widely used cancer biomarker that is PSA-KLK3, with millions of tests performed annually worldwide. The present report provides an overview of the biomarker potential of the extended KLK family (KLK1-KLK15) in various disease settings and envisages approaches that could lead to additional KLK-driven applications in future molecular diagnostics. Particular focus is given on the inclusion of KLKs into multifaceted cancer biomarker panels that provide enhanced diagnostic, prognostic and/or predictive accuracy in several human malignancies. Such panels have been described so far for prostate, ovarian, lung and colorectal cancers. The role of KLKs as biomarkers in non-malignant disease settings, such as Alzheimer's disease and multiple sclerosis, is also commented upon. Predictions are given on the challenges and future directions regarding clinically oriented KLK research.

KEYWORDS: biomarker panel • cancer diagnosis • cancer prognosis • kallikrein-related peptidases • KLK • molecular tumor marker • neurodegenerative diseases • ovarian cancer • prostate cancer • PSA

Kallikrein-related peptidases: serine proteases with extensive implication in human (patho)physiology & unique biomarker capabilities

Prostate-specific antigen (PSA) or kallikrein-related peptidase 3 (KLK3) is the most widely recognized member of the tissue kallikrein and kallikrein-related peptidases gene family (*KLKs*) [1]. PSA–KLK3 has been broadly used as a prostate cancer biomarker for almost three decades [2–6].

KLKs, located on chromosome 19q13.3–q13.4, comprise 15 gene members (*KLK1*–*KLK15*) [1] that have been suggested, in a plethora of studies, as promising disease biomarkers [7–9]. Efforts have also been made in order to evaluate the therapeutic potential of KLKs through various approaches that include engineered inhibitors, prodrugs activation and KLK-driven immunotherapy [10,11].

Perhaps, the continuously reported broad role of KLKs as biomarkers is a reflection of the important functions they serve in human physiology. KLKs share notable similarities at the levels of gene/protein structure (five coding exons that encode for inactive zymogens), protein activity (trypsin or chymotrypsin like) and biological regulation of their gene expression (by steroid hormones, methylation, histone modification, miRNAs) and enzyme activity (cleavage activation, inhibition by endogenous molecules) [12–14]. Under physiological conditions, KLKs act on their substrates, which include growth factors, hormones, proteases and cell signaling molecules, in order to orchestrate important body functions such as skin homeostasis, blood pressure regulation, semen liquefaction and neuronal plasticity [13–16]. Looking at the other side of the coin, deregulation of KLK function can trigger the manifestation of severe diseases. KLKs have been primarily studied as molecules that can influence the initiation and progression of human malignancies [9–12,16]. Nonetheless, their involvement has also been thoroughly investigated in neurodegenerative/neuroinflammatory disorders [15–18] and, particularly, skin diseases [19–22].

The present report provides an overview of the KLK biomarker potential in various disease settings and envisages approaches that increase

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the probabilities of introducing additional KLK-driven applications in future molecular diagnostics. Expected challenges and predicted directions in clinically oriented KLK research are also commented upon.

KLKs as cancer biomarkers: from PSA–KLK3 to the extended KLK family

Clinical oncology has been radically evolved after the incorporation of biological tumor markers in the management of human malignancies. Representative examples of the few tumor biomarkers that have succeeded in reaching routine clinical practice include the *HER2* gene amplification and OncotypeDx gene expression panel tests as predictive biomarkers for breast cancer, CA125 for ovarian cancer monitoring, *BRAF* mutation assessment for melanoma and PSA testing for prostate cancer screening and monitoring [23,24].

PSA: a successful, yet controversial, cancer biomarker

The influence of PSA testing on clinical decision making, both in a positive and negative sense, is so unique that in the history of oncology, it is logical to categorize prostate cancer management in a pre-PSA period, the current PSA period, as well as a post-PSA period, which is agonizingly anticipated [23,25].

The first studies describing the usefulness of PSA as a prostate cancer biomarker showed its utility as a disease monitoring and recurrence prediction tool and in 1986 received US FDA approval for this use [4,26]. The use of PSA measurements still remains the gold standard in defining biochemical relapse and thus disease recurrence [27]. However, after its initial approval, PSA testing was also applied for diagnostic purposes. In the following years, detection of prostate cancer increased spectacularly in the USA. The broad application of PSA testing as a screening strategy in asymptomatic men (FDA approved for this use in 1994) led to an impressive decrease in the cases diagnosed with advanced disease [28,29]. Nonetheless, all these important benefits came with a price. PSA serum levels are increased not only in clinically relevant prostate cancer, but also in every lesion that alters prostate gland's architecture including benign conditions and clinically indolent cancer. The widespread use of PSA testing led to more men with benign conditions being biopsied and to more men with no life-threatening tumors undergoing unnecessary radical treatment [23,30,31]. Two large-scale trials that investigated the effect of PSA screening *per se* in reducing prostate cancer mortality (Prostate, Lung, Colorectal, and Ovarian [PLCO] cancer screening trial and European Randomized Study of Screening for Prostate Cancer [ERSPC]) produced conflicting results. The PLCO trial [32], which was later criticized due to contamination of the unscreened arm, reported no benefit from screening, whereas ESPRC [33] reported a decrease in prostate cancer mortality [23,30]. The abovementioned data led the US Preventive Services Task Force to recommend against PSA screening, a decision that provoked extensive discussion and heavy criticism [23,34,35]. It seems that PSA testing as we know it may have gone full circle and its initial use for disease monitoring happens to be also the most suitable. The introduction of additional biomarkers that would limit the PSA-driven overdiagnosis/overtreatment and that could identify patients who will indeed benefit from hormonal and chemotherapy represents a clinical necessity. A systematic, multiphase procedure is needed in order for novel prostate cancer biomarkers to reach clinical practice. This carefully designed process should begin from the initial evidence-based discovery stage and continue with rigorous validation phases, including independent cohorts, retrospective and prospective studies, before finally reaching large-scale population trials [36].

Normalizing PSA to the prostate gland volume, determining changes in PSA measurements over time, measuring percentage of free PSA (%fPSA), complexed PSA, as well as the use of the more recently (2012) FDA-approved tests, such as the PCA3 urine test and the combined serum measurements of -2proPSA, free PSA and total PSA (calculated as prostate health index), have been proposed as next steps for improving the biomarker capabilities of PSA. Artificial neural networks, nomograms and logistic regression models can also help toward this direction [23,37–39]. Despite the reported improvements in the diagnostic performance of PSA by the aforementioned approaches, it is believed that a more radical paradigm shift is still needed.

As discussed in detail below (in 'KLKs in cancer biomarker panels: A more realistic and promising approach for routine clinical practice' section), the introduction of novel molecular markers belonging to the extended KLK family, through a biomarker panel strategy, could provide considerable improvements for prostate cancer screening.

Beyond PSA: the capacity of individual KLKs as biomarkers for human malignancies

Papers published on the association of KLKs with human malignancies (1933–2012) have been calculated to amount to the astonishing sum of 28,744 [40]. Every single member of the *KLK* gene family has been evaluated and proposed as a meaningful serum and/or tissue marker for at least one human malignancy [8,9].

Recently published review articles thoroughly describe the biomarker potential of the *KLK* gene family in urogenital and reproductive organ malignancies [41,42], gastrointestinal malignancies [43], lung, brain, head and neck cancers, and acute lymphoblastic leukemia [7–9]. A book volume dedicated to the biomarker potential of KLKs for cancer diagnosis, prognosis and treatment response prediction/monitoring has also been published [40]. A brief overview of this information is conveyed in Table 1.

The characterization of the extended cancer-related *KLK* family, including several alternative mRNA transcripts and protein isoforms, the comprehensive expression profiling of *KLKs* at different levels (mRNA, protein) and different disease settings (malignant and nonmalignant), as well as the significant improvements in the sensitivity and specificity of molecular

Table 1. The biomarker potential of individual KLKs in human malignancies.

Cancer type	Clinical relevance	Ref.
Prostate	Diagnosis: KLK2, KLK3, KLK11	[41]
	Prognosis/Prediction: KLK2, KLK3, KLK4, KLK5, KLK11, KLK14, KLK15	
Ovarian	Diagnosis: KLK5, KLK6, KLK8, KLK10, KLK11, KLK13, KLK14	[41,42,55]
	Prognosis/prediction: KLK4, KLK5, KLK6, KLK7, KLK8, KLK9, KLK10, KLK11, KLK13, KLK14, KLK15	
Breast	Diagnosis: KLK3, KLK5, KLK10, KLK14	[41,104]
	Prognosis/Prediction: KLK3, KLK4, KLK5, KLK7, KLK9, KLK10, KLK12, KLK13, KLK14, KLK15	
Lung	Diagnosis: KLK5, KLK7, KLK8, KLK10, KLK11, KLK12, KLK13, KLK14	[8,105]
	Prognosis: KLK6, KLK8, KLK11, KLK13	
Colorectal	Prognosis: KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13, KLK14	[43]
Gastric	Diagnosis: KLK6	[43,106]
	Prognosis: KLK6, KLK10, KLK11, KLK12, KLK13	
Endometrial	Diagnosis: KLK6, KLK10	[42]
	Prognosis: KLK6	
Cervical	KLK7 (increases with the severity of lesions)	[42]
Testicular	Prognosis: KLK5	[42]
Kidney	Prognosis: KLK6	[42]
Urinary bladder	KLK5, KLK6, KLK9 (increased in invasive tumors)	[107]
Head and neck	Prognosis: KLK4, KLK7, KLK11	[8]
Pancreatic	Prognosis: KLK6, KLK7, KLK10	[9,43]
Intracranial tumors	Prognosis: KLK6, KLK7	[9,108]

KLK: Kallikrein-related peptidases.

assays and reagents for KLK determination, raised expectations that the detailed information of *KLK* genes and proteins will trigger the identification of several potential tumor markers. The initial hopes and predictions have been fulfilled to a considerable extent, but still need to be realized into everyday clinical decision making.

KLKs in cancer biomarker panels: a more realistic & promising approach for routine clinical practice

A series of genomic and proteomic analyses have robustly demonstrated that cancer is an extremely heterogeneous disease in terms of morphological and biological features, clinical disease manifestation and progression, as well as response to currently available therapeutics [44]. Thus, it would be reasonable to foresee that no single biomarker will be ever capable of providing accurate enough information for cancer diagnosis, prognosis or treatment response prediction. The current trend for maximizing the clinical effectiveness of biomarkers, and thus compensating for their low incorporation rates into clinical practice, is the identification of combinatorial biomarker panels. KLKs have already been identified as valuable composites of such panels for prostate, ovarian, lung and colorectal cancers.

Perhaps, the most intriguing and well-validated data derived from a series of studies regarding the identification of a four-kallikrein serum panel consisting of total, free and intact PSA, as well as KLK2, for prostate cancer management. The above-mentioned 'kallikrein panel' can effectively predict the result of a prostate biopsy in both unscreened and previously screened men. The application of the 'kallikrein panel' could appropriately tackle the overdiagnosis and overtreatment complications deriving from PSA-based screening. It is a straightforward approach that makes use of routine laboratory tests and current standard of care in order to reduce the number of unnecessary prostate biopsies, and accompanying complications, with enhanced sensitivity (especially in high-grade tumors) and specificity (reduced detection rates of clinically indolent low-grade tumors) [45–47]. Moreover, statistical models based on the 'kallikrein panel' could effectively replace invasive procedures, such as transrectal ultrasonography and digital rectal examination, in predicting prostate biopsy outcome [48]. Another essential problem that the 'kallikrein panel' addresses is the economic impact of repeated unnecessary prostate biopsies that cannot be overlooked during the times of global economic crisis. A future application of the 'kallikrein panel' in routine diagnostics would reduce the unnecessary biopsies performed in

the USA up to 56%, without compromising standards of care; this translates to approximately US$1.12 billion savings per year for the US health care system [49].

Multivariate models and/or artificial neural networks combining serum KLK2, %fPSA and PSA levels could provide, to some extent, an improved discrimination between CaP and BPH patients. Similar approaches using serum KLK11, %fPSA, MIC-1, MIF, prostate volume or age can also enhance BPH and CaP discrimination. Logistic regression models have demonstrated the potential of serum IGF-1/fPSA ratio and PSP94–fPSA combination for the differential diagnosis of benign and malignant prostate tumors [38]. The incorporation of a number of single nucleotide polymorphisms (SNPs) in a nomogram also containing PSA, %fPSA, age and other clinical can improve the positive predictive value of PSA testing [50].

Regarding ovarian cancer, initial studies had demonstrated that serum KLK6 [51] and KLK10 [52] measurements can be used to enhance the diagnostic sensitivity of CA125 in early disease stages. A later study revealed improved diagnostic properties for the combination of serum KLK10 and CA125 over CA125 alone [53]. Additionally, combinations between serum KLK6 and KLK10 with CA125 and age provided improvements in the discriminatory accuracy for patients harboring ovarian tumors compared with single marker use [54]. A panel of eight KLKs (KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK13 and KLK14) determined in effusion samples produces remarkable areas under the curve (AUCs) of 0.99 and 0.96 for the discrimination of cancer from benign cases and ovarian cancer from other malignancies, respectively [55]. Performed in tissue samples, a panel of *KLK6*, *KLK13* and *MUC16* (encoding for CA125) mRNA levels increases the detection sensitivity for early stage ovarian cancer and the negative predictive value compared with *MUC16* alone [56]. In cytosolic extracts, the combination of CA125, B7-H4, KLK4, KLK5, KLK7, KLK8 and KLK11 and the combination of CA125, KLK8, KLK10 and KLK13 protein levels can effectively distinguish between ovarian cancer and benign tumors, and between primary ovarian cancer and primary tumors metastatic to the ovary, respectively [57].

KLK-based biomarker panels with prognostic significance can also be proven extremely helpful in ovarian cancer decision making. A combination of KLK6 and KLK13 tissue protein levels, ascites volume and nuclear grade can accurately predict (AUC = 0.833) the presence of residual tumor after surgery and thus instruct the administration of preoperative chemotherapy [58]. The combined determination of KLK13, KLK6, KLK8 protein levels in ovarian cytosolic extracts, disease stage and debulking status can efficiently identify patients' response to chemotherapy (AUC = 0.91). Similarly, impressive predictive capabilities have been identified regarding 1-year progression-free survival (AUC = 0.90) for a combination of KLK6, KLK8, KLK11, KLK1 and clinical variables (i.e., stage, debulking success, chemotherapy response), and 5-year progression-free survival (AUC = 0.93) for a combination of KLK6, KLK7, KLK11, KLK14, B7-H4 and clinical variables [57]. Serum-based multiparametric panels have also been constructed and could offer valuable prognostic information regarding 1-year survival (KLK7, KLK10, B7-H4 and Spondin-2), 1-year disease progression (CA125, KLK7, KLK8 and Spondin-2) and chemotherapy response (CA125, KLK5 and KLK7) [59].

In non-small-cell lung cancer (NSCLC), the study of Planque *et al*. has demonstrated the diagnostic capabilities (AUC = 0.90) of combined KLK4, KLK8, KLK10, KLK11, KLK12, KLK13 and KLK14 serum measurements for the discrimination between NSCLC patients and healthy individuals. Another model that includes serum KLK8, KLK11, KLK12, KLK13, KLK1 as well as gender and smoking produced an AUC of 0.92 [60]. Several combinatory assessments of *KLK* mRNA expression levels can provide important prognostic information regarding the overall survival (OS) of NSCLC patients. The *KLK8* alternative transcript 4/*KLK11* mRNA ratio in lung cancer is a strong independent predictor of poor OS, showing better prognostic performance even from clinical stage [61].

In colorectal cancer, the combination of tissue protein levels of KLK14, and KLK8, KLK10, KLK14, KLK15, with clinical parameters (age and TNM stage) leads to an increased predictive potential of 1-year and 5-year OS, respectively, compared with clinical parameters alone [62].

Regulation of KLK expression by miRNAs & DNA methylation: epigenetic mechanisms with great translational potential

Determinations of DNA methylation status and miRNA expression levels are regarded as valuable biological tumor markers. They can be easily assessed in body fluids, such as blood or urine, as well as in archival material (formalin-fixed paraffin-embedded tissues) that is accompanied by invaluable clinical follow-up information [63–65].

The miRNA–KLK axis of interaction has been described so far in prostate cancer [66], ovarian cancer [67] and renal cell carcinoma [68]. Several miRNAs that are deregulated in the aforementioned malignancies are predicted to target *KLKs*. The experimentally validated interactions include those between miR-331-3p and *KLK4*, miR-143 and *KLK10*, miR-224 and both *KLK1* and *KLK10*, let-7f and both *KLK6* and *KLK10*, miR-516a and *KLK10*, as well as between members of the miR-99 family and *KLK3* [14,69]. In ovarian and prostate cancers, a significant negative correlation between the expression levels of miRNAs and their target *KLKs* has been documented [14,66,69]. For example, a negative correlation between the expression levels of miR-224 and its predicted target *KLK15* has been reported in prostate cancer [69]. Interestingly, both *KLK15* and miR-224 have been repeatedly proposed by independent researchers as important biomarkers of unfavorable and favorable, respectively, prognosis for prostate cancer patients [70–73]. We believe that the combination of miRNA and *KLK* expression into informative

diagnostic/prognostic scores can constitute a novel approach in stratifying and translating the heterogeneity of human malignancies to meaningful clinical information.

The *KLK* locus contains multiple CpG islands, and DNA methylation represents a suggested mechanism of *KLK* transcriptional regulation for certain gene members (e.g., *KLK6*, *KLK10*, *KLK1*, *KLK11*, *KLK12* and *KLK13*). The decreased expression of certain *KLKs* observed in some human malignancies (e.g., *KLK6* in breast cancer, *KLK10* in NSCLC) is closely associated with CpG island hypermethylation [14,74]. *KLK10* exon 3 methylation is associated with poor prognosis in acute lymphoblastic leukemia and breast cancer, while it is also considered as an indicator of advanced disease in NSCLC [14]. A recent study shows that low *KLK10* methylation levels are associated with biochemical relapse in prostate cancer patients [75].

There is even more to KLKs than their tumor marker potential: the role of KLKs as biomarkers in nonmalignant diseases

Without doubt, most of the studies regarding the biomarker capacity of KLKs concern human malignancies. Nonetheless, studies addressing the potential role of KLKs in the molecular diagnostics of nonmalignant diseases are also intriguing.

The deregulation of KLK levels has been comprehensively reported in nonmalignant diseases of the CNS [11,76]. Serum and/or cerebrospinal fluid (CSF) KLK levels could be considered as biomarkers for major CNS diseases.

In Alzheimer's disease (AD), CSF levels of KLK10 increase, while those of KLK7 decrease. Lower KLK7 CSF levels are associated with the presence of ApoE4 alleles, a risk factor of AD [77]. Both whole blood and CSF levels of KLK6 were initially reported to be increased in AD patients compared with normal controls [78]. Contrariwise, it has been recently shown that patients with synucleinopathy showed lower KLK6 CSF levels compared with controls and AD patients; no difference was found in KLK6 CSF levels between AD and control samples in the same study [79]. KLK6 CSF and plasma levels are positively correlated with age in normal individuals, and this association is lost [80] or even inverted [81] in AD patients. Decreased KLK6 CSF levels have been proposed as a risk factor for AD development [80]. Interestingly, KLK6 plasma concentrations can be used for the discrimination between AD patients and individuals without neurodegenerative dementia [81], as well as for calculating the risk for progression of mild cognitive impairment to dementia with vascular component or AD [82]. Furthermore, patients with frontotemporal dementia exhibit decreased KLK6, KLK7 and KLK10 CSF levels compared with control subjects [77].

Serum KLK6 levels are increased after traumatic brain injury [83] and in postpolio syndrome [84], whereas they are decreased in a life-threatening condition known as aneurysmal subarachnoid hemorrhage, with the lowest levels measured in patients with worse outcome [85].

Aberrant KLK levels are also found in multiple sclerosis (MS). Interestingly, KLK6 participates directly in the pathogenesis of MS by cleaving basic myelin proteins [17,76]. An initial study has shown that serum KLK1 and KLK6 levels are elevated in MS patients and are associated with secondary progressive MS and expanded disability status scale scores [86]. Patients diagnosed with advanced MS exhibit elevated KLK6 CSF levels compared with neurological controls [87]. On the contrary, three recent proteomic studies have shown that KLK6 is significantly decreased in the CSF of patients with relapsing–remitting MS compared with other neurological disease controls [88–90]. Significantly increased anti-KLK11 antibody levels (82% sensitivity, 94% specificity) are found in the serum of patients diagnosed with Sjögren's syndrome, another autoimmune-mediated disease [91].

One of the well-studied physiological roles of KLKs is their central contribution in maintaining skin homeostasis and desquamation (mainly for KLK5, KLK7 and KLK14). Studies on Netherton syndrome, a rare genetic skin disease linked with mutations in the *SPINK5* gene encoding for the inhibitor LEKTI that blocks KLK activity, have shown that the lack of LEKTI causes KLK overactivation, over-desquamation of corneocytes, inflammation and ultimately severe skin dysfunction [19–22,92]. Regarding the biomarker capacity of KLKs in skin diseases, they may hold promise as biomarkers for psoriasis. KLK6 and KLK8 are increased in psoriatic arthritis synovial fluid compared with noninflammatory osteoarthritis. KLK8 serum levels are also elevated in psoriatic disease and are independently associated with cutaneous psoriasis activity, but not with arthritic disease activity. Consequently, KLK8 could constitute a novel surrogate molecular marker for estimating cutaneous psoriasis severity and monitoring therapeutic response [93]. KLK10 and KLK13 serum levels are also correlated with the severity of skin lesions in psoriasis. Interestingly, KLK5 and KLK11 concentrations are lower in the serum of psoriasis vulgaris and arthropathic psoriasis patients compared with normal individuals and are significantly decreased after therapy [94]. As far as atopic dermatitis is concerned, serum KLK8 levels are increased, whereas KLK5 and KLK11 levels are decreased [95]; a *KLK7* polymorphism (AACC insertion in the 3′-UTR) has also been associated with this disease [96].

KLK SNPs: biomarker potential deriving from yet another facet of KLKs association with human pathology

A plethora of data demonstrates the linkage between SNPs within the *KLK* locus and a wide variety of diseases [96]. In the nonmalignant setting, *KLK1* SNPs are associated with hypertension (rs3212816, rs5517), cerebral hemorrhage (rs5517) and cardiovascular disease (rs5515), a *KLK2* SNP is related to male infertility (rs2664155), a *KLK4* mutation has been identified as causing event of a tooth disorder (mutation: g.2142 G > A, Trp153Stop) and certain *KLK8* SNPs have been linked with intracranial aneurysm (rs1722561, rs1701946) [96] and bipolar disorder (rs1612902) [97]. Prostate cancer has been the cornerstone of *KLK*-related SNPs research, perhaps due to the importance of PSA. *KLK2*

(rs198977, rs2664155, rs1506684), *KLK3* (e.g., rs266882, rs1058205, rs266870, rs2659122), *KLK4* (e.g., rs7248321, rs1654551, rs1701927, rs806019) *KLK10* (rs3745535) and *KLK12* (rs3865443) SNPs have been associated with prostate cancer risk, *KLK3* (e.g., rs266882, rs2735839, rs17632542), *KLK4* (rs198968), *KLK14* (rs17728459, rs35287116) and *KLK15* (rs2659056) SNPs with disease aggressiveness, two *KLK3* SNPs (rs61752561, rs2735839) with patients' survival and a *KLK2* SNP (rs198977) with biochemical recurrence. Likewise, KLK2 (rs198977) and *KLK4* (rs806019) SNPs have been associated with breast cancer risk, a *KLK3* SNP was related to less aggressive breast cancer (rs11575894) as well as *KLK3* (rs11084033) and *KLK15* (rs266851) SNPs were linked with ovarian cancer survival [96,98–100].

Even though individual *KLK* SNPs may not be likely to be used as a standalone test in future molecular diagnostics, the multifactorial approach seems, once again, to be more realistic. The combination of *KLK* SNPs with these of other loci and/or with other conventional clinicopathological data may form the basis of a rapid, easily performed DNA-based clinical test. Toward this direction, it has already been shown that a *KLK2* SNP (rs198977) can be combined with clinical data or serum KLK2 levels in order to improve the prediction of biochemical relapse [96] or to enhance prostate cancer detection [100], respectively. Other *KLK* SNPs can also aid in prostate cancer risk prediction and therapeutic decision making though their incorporation into multifactorial models [101].

Expert commentary

The use of PSA measurements as a prostate cancer biomarker was a major breakthrough for the practice of oncology. Nonetheless, its extensive use as screening tool in asymptomatic men introduced complexities in prostate cancer management. Without intending to overlook the benefits that PSA has provided regarding the detection of prostate cancer in early stages, it should be noted that PSA-driven overdiagnosis and overtreatment are issues that still remain to be tackled [23,30]. The current challenge is to identify multifactorial biomarker panels that will provide clear advantages over individual biomarkers such as PSA. The application of KLK-based biomarker panels could assist in the decision-making processes primarily for prostate cancer and, in the long term, for other malignancies as well.

Data from the 'four-kallikrein panel' approach are very encouraging toward the reduction of unnecessary prostate biopsies [45–49]. Ovarian, lung and colorectal cancer management could also be enhanced by multifactorial biomarker panels that include multiple KLK members [54–62]. The amount of KLK-derived information that can be combined is enormous and has been made available through decades of, still ongoing, translational research. *KLK* expression (mRNA and protein levels), *KLK*-targeting miRNAs, SNPs found within the *KLK* locus and the methylation status of *KLK* genes are examples of detailed molecular data that could be combined with traditional clinicopathological factors. This multifaceted approach can enhance the diagnostic, prognostic, predictive and/or treatment monitoring potency of the one-sided procedures that are currently followed throughout cancer patients' management. Well-designed and established assays are already available for KLK assessment at the protein (ELISA, immunohistochemistry), RNA (qPCRs for mRNA and *KLK*-targeting miRNAs) and DNA (quantitative methylation methods, sequencing for SNPs) levels. These easily performed assays can be cost-effectively used in routine clinical practice. Interestingly, KLK levels can be also measured in the serum and CSF of patients suffering from nonmalignant diseases. This has opened new opportunities for the consideration of KLKs as biomarkers for major neurodegenerative and neuroinflammatory diseases such as AD and MS [78,81,86,87].

Five-year view

It is rather unlikely that any single-biomarker test could address the heterogeneity observed, in terms of biological and clinical manifestation, in most human malignancies. Combining multiple biomarkers with conventional, yet strong, indicators can significantly improve the accuracy of molecular diagnostics. The FDA clearance of the multifactorial ROMA score and OVA1 panel for ovarian cancer enhances this notion further [24].

The significance of several *KLK* members as individual biomarkers has been corroborated by independent research groups [7,8,12,41,42]. Nonetheless, the need for multicentric large-scale validation still exists. We believe that there is a niche for additional KLKs in the treatment decision making of cancer patients and that this can be achieved through their integration in well-defined biomarker panels. Based on the repeatedly validated and straightforward results that have been reported [45–49], it is highly probable that the 'four-kallikrein panel' will be eventually introduced in clinical practice for prostate cancer. The recent FDA approvals for prostate health index (based on serum-2pro-, total- and free PSA measurements) and urinary PCA3 score highlight the intense medical need for improving decisions regarding prostate cancer management.

Current and future trends in KLK research, such as the elucidation of miRNA–*KLKs* axis of interaction, deciphering the *KLK*-methylation patterns, characterization of *KLK* SNPs, discovery of novel *KLK* mRNA transcripts, determination of glycosylated KLK isoforms – especially for KLK3 [102] and KLK6 [103] – could help in transforming the envisagement of the incorporation of additional KLKs in future molecular diagnostics into a clinical reality.

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Key issues

- The Kallikrein-related peptidase (*KLK*) gene family encodes for 15 secreted serine proteases, many of which have been described as cancer-related molecules.
- KLK3 (also known as prostate-specific antigen [PSA]) represents the most renowned member of the *KLK* family. The wide application of PSA as a biomarker for prostate cancer screening has provided notable benefits, such as the detection of prostate cancer at early stages and a, much debated, decrease in mortality rates.
- The benefits of PSA testing came with the complications of overdiagnosis and overtreatment. Thus, novel prostate cancer biomarkers should be introduced in order to reduce the amount of unnecessary biopsies.
- Individual KLK members have been described as biomarkers for the majority of human malignancies including prostate, ovarian, breast, lung and colorectal cancers.
- The incorporation of KLKs into multifaceted biomarker panels provides enhanced diagnostic, prognostic and predictive accuracy in several human malignancies. The most successful example is that of the 'four-kallikrein panel' for prostate cancer, which has great dynamics for introduction into future clinical practice.
- Multifactorial KLK-driven biomarker panels have also been proposed for ovarian, lung and colorectal cancer decision making.
- KLK-derived information that could be combined in biomarker panels includes data from the *KLK* expression profiling (mRNA and/or protein levels), *KLK*-targeting miRNAs, SNPs found within the *KLK* locus and methylation status of *KLK* genes.
- KLKs are also regarded as promising biomarkers for nonmalignant diseases such as Alzheimer's disease and multiple sclerosis.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Yousef GM, Chang A, Scorilas A, Diamandis EP. Genomic organization of the human kallikrein gene family on chromosome 19q13.3-q13.4. Biochem Biophys Res Commun 2000;276(1):125-33

• **The first detailed description of the extended human KLK gene locus.**

2. Wang MC, Valenzuela LA, Murphy GP, Chu TM. Purification of a human prostate specific antigen. Invest Urol 1979;17(2): 159-63
3. Kuriyama M, Wang MC, Papsidero LD, et al. Quantitation of prostate-specific antigen in serum by a sensitive enzyme immunoassay. Cancer Res 1980;40(12): 4658-62
4. Stamey TA, Yang N, Hay AR, et al. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 1987;317(15):909-16
5. Lilja H, Christensson A, Dahlen U, et al. Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. Clin Chem 1991;37(9): 1618-25
6. Stenman UH, Leinonen J, Alfthan H, et al. A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. Cancer Res 1991;51(1):222-6
7. Emami N, Diamandis EP. Utility of kallikrein-related peptidases (KLKs) as cancer biomarkers. Clin Chem 2008;54(10): 1600-7
8. Kontos CK, Scorilas A. Kallikrein-related peptidases (KLKs): a gene family of novel cancer biomarkers. Clin Chem Lab Med 2012;50(11):1877-91
9. Mavridis K, Scorilas A. Prognostic value and biological role of the kallikrein-related peptidases in human malignancies. Future Oncol 2010;6(2):269-85
10. Mavridis K, Avgeris M, Scorilas A. Targeting kallikrein-related peptidases in prostate cancer. Expert Opin Ther Targets 2014;18(4):365-83

• **A recent overview of the therapeutic relevance of KLKs in prostate cancer and other diseases.**

11. Sotiropoulou G, Pampalakis G. Targeting the kallikrein-related peptidases for drug development. Trends Pharmacol Sci 2012; 33(12):623-34
12. Lawrence MG, Lai J, Clements JA. Kallikreins on steroids: structure, function, and hormonal regulation of prostate-specific antigen and the extended kallikrein locus. Endocr Rev 2010;31(4):407-46
13. Emami N, Diamandis EP. New insights into the functional mechanisms and clinical applications of the kallikrein-related peptidase family. Mol Oncol 2007;1(3): 269-87
14. Pasic MD, Olkhov E, Bapat B, Yousef GM. Epigenetic regulation of kallikrein-related peptidases: there is a whole new world out there. Biol Chem 2012;393(5): 319-30

•• **A concise description of the emerging role of miRNAs and epigenetics in KLK-related research.**

15. Sotiropoulou G, Pampalakis G, Diamandis EP. Functional roles of human kallikrein-related peptidases. J Biol Chem 2009;284(48):32989-94

•• **A well-described overview of the broad roles of KLKs in human physiology.**

16. Borgono CA, Michael IP, Diamandis EP. Human tissue kallikreins: physiologic roles and applications in cancer. Mol Cancer Res 2004;2(5):257-80

17. Scarisbrick IA, Yoon H, Panos M, et al. Kallikrein 6 regulates early CNS demyelination in a viral model of multiple sclerosis. Brain Pathol 2012;22(5):709-22
18. Yousef GM, Kishi T, Diamandis EP. Role of kallikrein enzymes in the central nervous system. Clin Chim Acta 2003;329(1-2):1-8
19. Brattsand M, Egelrud T. Purification, molecular cloning, and expression of a human stratum corneum trypsin-like serine protease with possible function in desquamation. J Biol Chem 1999;274(42): 30033-40
20. Brattsand M, Stefansson K, Lundh C, et al. A proteolytic cascade of kallikreins in the stratum corneum. J Invest Dermatol 2005; 124(1):198-203
21. Caubet C, Jonca N, Brattsand M, et al. Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/ KLK7/hK7. J Invest Dermatol 2004;122(5): 1235-44
22. Deraison C, Bonnart C, Lopez F, et al. LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. Mol Biol Cell 2007;18(9): 3607-19
23. Prensner JR, Rubin MA, Wei JT, Chinnaiyan AM. Beyond PSA: the next generation of prostate cancer biomarkers. Sci Transl Med 2012;4(127):127rv123

•• **A comprehensive review that successfully conveys the main aspects of prostate-specific antigen testing.**

24. Leung F, Diamandis EP, Kulasingam V. From bench to bedside: discovery of ovarian cancer biomarkers using high-throughput technologies in the past decade. Biomark Med 2012;6(5):613-25
25. Schroder FH. Landmarks in prostate cancer screening. BJU Int 2012(110 Suppl 1):3-7
26. Stamey TA, Graves HC, Wehner N, et al. Early detection of residual prostate cancer after radical prostatectomy by an ultrasensitive assay for prostate specific antigen. J Urol 1993;149(4):787-92
27. Boccon-Gibod L, Djavan WB, Hammerer P, et al. Management of prostate-specific antigen relapse in prostate cancer: a European Consensus. Int J Clin Pract 2004;58(4):382-90
28. Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med 1991;324(17): 1156-61
29. Potosky AL, Feuer EJ, Levin DL. Impact of screening on incidence and mortality of prostate cancer in the United States. Epidemiol Rev 2001;23(1):181-6
30. Marta GN, Hanna SA, Fernandes da Silva JL, Carvalho Hde A. Screening for prostate cancer: an updated review. Expert Rev Anticancer Ther 2013; 13(1):101-8
31. Vickers AJ, Roobol MJ, Lilja H. Screening for prostate cancer: early detection or overdetection? Annu Rev Med 2012;63: 161-70
32. Andriole GL, Crawford ED, Grubb RL 3rd, et al. Mortality results from a randomized prostate-cancer screening trial. N Engl J Med 2009;360(13):1310-19
33. Schroder FH, Hugosson J, Roobol MJ, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med 2009;360(13):1320-8
34. Carter HB. American Urological Association (AUA) guideline on prostate cancer detection: process and rationale. BJU Int 2013;112(5):543-7
35. Chou R, Croswell JM, Dana T, et al. Screening for prostate cancer: a review of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med 2011;155(11): 762-71
36. Prensner JR, Chinnaiyan AM, Srivastava S. Systematic, evidence-based discovery of biomarkers at the NCI. Clin Exp Metastasis 2012;29(7):645-52
37. Melichar B. PSA, PCA3 and the phi losophy of prostate cancer management. Clin Chem Lab Med 2013;51(4):707-12
38. Stephan C, Rittenhouse H, Cammann H, et al. New markers and multivariate models for prostate cancer detection. Anticancer Res 2009;29(7):2589-600
39. Stephan C, Jung K, Semjonow A, et al. Comparative assessment of urinary prostate cancer antigen 3 and TMPRSS2:ERG gene fusion with the serum [-2]proprostate-specific antigen-based prostate health index for detection of prostate cancer. Clin Chem 2013;59(1):280-8
40. Schmitt M, Sommerhoff CP, Fritz H, Magdolen V. Introduction to Volume 2: Kallikrein-related peptidases. novel cancer-related biomarkers. De Gruyter; Berlin, Germany: 2012
41. Avgeris M, Mavridis K, Scorilas A. Kallikrein-related peptidases in prostate, breast, and ovarian cancers: from pathobiology to clinical relevance. Biol Chem 2012;393(5):301-17
42. Dorn J, Bayani J, Yousef GM, et al. Clinical utility of kallikrein-related peptidases (KLK) in urogenital malignancies. Thromb Haemost 2013; 110(3):408-22
43. Kontos CK, Mavridis K, Talieri M, Scorilas A. Kallikrein-related peptidases (KLKs) in gastrointestinal cancer: mechanistic and clinical aspects. Thromb Haemost 2013;110(3):450-7
44. Nowsheen S, Aziz K, Panayiotidis MI, Georgakilas AG. Molecular markers for cancer prognosis and treatment: have we struck gold? Cancer Lett 2012;327(1-2): 142-52
45. Gupta A, Roobol MJ, Savage CJ, et al. A four-kallikrein panel for the prediction of repeat prostate biopsy: data from the European Randomized Study of Prostate Cancer screening in Rotterdam, Netherlands. Br J Cancer 2010;103(5): 708-14
46. Vickers A, Cronin A, Roobol M, et al. Reducing unnecessary biopsy during prostate cancer screening using a four-kallikrein panel: an independent replication. J Clin Oncol 2010;28(15): 2493-8
47. Vickers AJ, Cronin AM, Roobol MJ, et al. A four-kallikrein panel predicts prostate cancer in men with recent screening: data from the European Randomized Study of Screening for Prostate Cancer, Rotterdam. Clin Cancer Res 2010;16(12): 3232-9
48. Carlsson SV, Peltola MT, Sjoberg D, et al. Can one blood draw replace transrectal ultrasonography-estimated prostate volume to predict prostate cancer risk? BJU Int 2013;112(5):602-9

• **A recent study describing the direct clinical applicability of the kallikrein panel.**

49. Voigt JD, Zappala SM, Vaughan ED, Wein AJ. The Kallikrein Panel for prostate cancer screening: its economic impact. Prostate 2014;74(3):250-9

•• **A very interesting article describing the economic benefits of switching from prostate-specific antigen to kallikrein panel testing.**

50. Nam RK, Zhang WW, Trachtenberg J, et al. Utility of incorporating genetic variants for the early detection of prostate cancer. Clin Cancer Res 2009;15(5): 1787-93
51. Diamandis EP, Scorilas A, Fracchioli S, et al. Human kallikrein 6 (hK6): a new potential serum biomarker for diagnosis and

prognosis of ovarian carcinoma. J Clin Oncol 2003;21(6):1035-43

52. Luo LY, Katsaros D, Scorilas A, et al. The serum concentration of human kallikrein 10 represents a novel biomarker for ovarian cancer diagnosis and prognosis. Cancer Res 2003;63(4):807-11
53. El Sherbini MA, Sallam MM, Shaban EA, El-Shalakany AH. Diagnostic value of serum kallikrein-related peptidases 6 and 10 versus CA125 in ovarian cancer. Int J Gynecol Cancer 2011;21(4): 625-32
54. Koh SC, Huak CY, Lutan D, et al. Combined panel of serum human tissue kallikreins and CA-125 for the detection of epithelial ovarian cancer. J Gynecol Oncol 2012;23(3):175-81
55. Shih Ie M, Salani R, Fiegl M, et al. Ovarian cancer specific kallikrein profile in effusions. Gynecol Oncol 2007;105(2): 501-7
56. White NM, Mathews M, Yousef GM, et al. Human kallikrein related peptidases 6 and 13 in combination withCA125 is a more sensitive test for ovarian cancer than CA125 alone. Cancer Biomark 2009;5(6): 279-87
57. Zheng Y, Katsaros D, Shan SJ, et al. A multiparametric panel for ovarian cancer diagnosis, prognosis, and response to chemotherapy. Clin Cancer Res 2007; 13(23):6984-92
58. Dorn J, Schmitt M, Kates R, et al. Primary tumor levels of human tissue kallikreins affect surgical success and survival in ovarian cancer patients. Clin Cancer Res 2007; 13(6):1742-8
59. Oikonomopoulou K, Li L, Zheng Y, et al. Prediction of ovarian cancer prognosis and response to chemotherapy by a serum-based multiparametric biomarker panel. Br J Cancer 2008;99(7):1103-13
60. Planque C, Li L, Zheng Y, et al. A multiparametric serum kallikrein panel for diagnosis of non-small cell lung carcinoma. Clin Cancer Res 2008;14(5): 1355-62
61. Planque C, Choi YH, Guyetant S, et al. Alternative splicing variant of kallikrein-related peptidase 8 as an independent predictor of unfavorable prognosis in lung cancer. Clin Chem 2010; 56(6):987-97
62. Talieri M, Li L, Zheng Y, et al. The use of kallikrein-related peptidases as adjuvant prognostic markers in colorectal cancer. Br J Cancer 2009;100(10):1659-65
63. Kotorashvili A, Ramnauth A, Liu C, et al. Effective DNA/RNA co-extraction for analysis of microRNAs, mRNAs, and genomic DNA from formalin-fixed paraffin-embedded specimens. PLoS One 2012;7(4): e34683
64. Allegra A, Alonci A, Campo S, et al. Circulating microRNAs: new biomarkers in diagnosis, prognosis and treatment of cancer (review). Int J Oncol 2012;41(6):1897-912
65. Liu A, Xu X. MicroRNA isolation from formalin-fixed, paraffin-embedded tissues. Methods Mol Biol 2011;724:259-67
66. White NM, Youssef YM, Fendler A, et al. The miRNA-kallikrein axis of interaction: a new dimension in the pathogenesis of prostate cancer. Biol Chem 2012;393(5): 379-89
67. White NM, Chow TF, Mejia-Guerrero S, et al. Three dysregulated miRNAs control kallikrein 10 expression and cell proliferation in ovarian cancer. Br J Cancer 2010;102(8):1244-53
68. White NM, Bui A, Mejia-Guerrero S, et al. Dysregulation of kallikrein-related peptidases in renal cell carcinoma: potential targets of miRNAs. Biol Chem 2010;391(4):411-23
69. Yousef GM, White NM. Kallikrein-related peptidases. Volume 2, Chapter 8 microRNAs: a new control mechanism for kallikrein-related peptidases in kidney and other cancers. De Gruyter; Berlin, Germany: 2012. p. 167-82
70. Mavridis K, Stravodimos K, Scorilas A. Quantified KLK15 gene expression levels discriminate prostate cancer from benign tumors and constitute a novel independent predictor of disease progression. Prostate 2013;73(11):1191-201
71. Mavridis K, Stravodimos K, Scorilas A. Downregulation and prognostic performance of microRNA 224 expression in prostate cancer. Clin Chem 2013;59(1): 261-9
72. Rabien A, Fritzsche FR, Jung M, et al. KLK15 is a prognostic marker for progression-free survival in patients with radical prostatectomy. Int J Cancer 2010; 127(10):2386-94
73. Lin ZY, Huang YQ, Zhang YQ, et al. MicroRNA-224 inhibits progression of human prostate cancer by downregulating TRIB1. Int J Cancer 2014;135(3):541-50
74. Pampalakis G, Diamandis EP, Sotiropoulou G. The epigenetic basis for the aberrant expression of kallikreins in human cancers. Biol Chem 2006;387(6):795-9
75. Olkhov-Mitsel E, Van der Kwast T, Kron KJ, et al. Quantitative DNA methylation analysis of genes coding for kallikrein-related peptidases 6 and 10 as biomarkers for prostate cancer. Epigenetics 2012;7(9):1037-45
76. Bayani J, Diamandis EP. The physiology and pathobiology of human kallikrein-related peptidase 6 (KLK6). Clin Chem Lab Med 2012;50(2):211-33
77. Diamandis EP, Scorilas A, Kishi T, et al. Altered kallikrein 7 and 10 concentrations in cerebrospinal fluid of patients with Alzheimer's disease and frontotemporal dementia. Clin Biochem 2004;37(3): 230-7
78. Diamandis EP, Yousef GM, Petraki C, Soosaipillai AR. Human kallikrein 6 as a biomarker of alzheimer's disease. Clin Biochem 2000;33(8):663-7
79. Wennstrom M, Surova Y, Hall S, et al. Low CSF levels of both alpha-synuclein and the alpha-synuclein cleaving enzyme neurosin in patients with synucleinopathy. PLoS One 2013;8(1):e53250
80. Mitsui S, Okui A, Uemura H, et al. Decreased cerebrospinal fluid levels of neurosin (KLK6), an aging-related protease, as a possible new risk factor for Alzheimer's disease. Ann N Y Acad Sci 2002;977: 216-23
81. Menendez-Gonzalez M, Castro-Santos P, Suarez A, et al. Value of measuring plasmatic levels of neurosin in the diagnosis of Alzheimer's disease. J Alzheimers Dis 2008;14(1):59-67
82. Menendez-Gonzalez M, Castro-Santos P, Calatayud MT, et al. Plasmatic level of neurosin predicts outcome of mild cognitive impairment. Int Arch Med 2008; 1(1):11
83. Phipps HW, Longo LM, Blaber SI, et al. Kallikrein-related peptidase 6: a biomarker for traumatic brain injury in the rat. Brain Inj 2013;27(13-14):1698-706
84. Gonzalez H, Ottervald J, Nilsson KC, et al. Identification of novel candidate protein biomarkers for the post-polio syndrome - implications for diagnosis, neurodegeneration and neuroinflammation. J Proteomics 2009;71(6):670-81
85. Martinez-Morillo E, Diamandis A, Romaschin AD, Diamandis EP. Kallikrein 6 as a serum prognostic marker in patients with aneurysmal subarachnoid hemorrhage. PLoS One 2012;7(9):e45676
86. Scarisbrick IA, Linbo R, Vandell AG, et al. Kallikreins are associated with secondary progressive multiple sclerosis and promote neurodegeneration. Biol Chem 2008;389(6): 739-45

87. Hebb AL, Bhan V, Wishart AD, et al. Human kallikrein 6 cerebrospinal levels are elevated in multiple sclerosis. Curr Drug Discov Technol 2010;7(2):137-40

88. Kroksveen AC, Guldbrandsen A, Vedeler C, et al. Cerebrospinal fluid proteome comparison between multiple sclerosis patients and controls. Acta Neurol Scand Suppl 2012(195):90-6

89. Kroksveen AC, Aasebo E, Vethe H, et al. Discovery and initial verification of differentially abundant proteins between multiple sclerosis patients and controls using iTRAQ and SID-SRM. J Proteomics 2013;78:312-25

90. Harris VK, Diamanduros A, Good P, et al. Bri2-23 is a potential cerebrospinal fluid biomarker in multiple sclerosis. Neurobiol Dis 2010;40(1):331-9

91. El Annan J, Jiang G, Wang D, et al. Elevated immunoglobulin to tissue KLK11 in patients with Sjogren syndrome. Cornea 2013;32(5):e90-3

92. Chavanas S, Bodemer C, Rochat A, et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. Nat Genet 2000;25(2):141-2

93. Eissa A, Cretu D, Soosaipillai A, et al. Serum kallikrein-8 correlates with skin activity, but not psoriatic arthritis, in patients with psoriatic disease. Clin Chem Lab Med 2013;51(2):317-25

94. Komatsu N, Saijoh K, Kuk C, et al. Aberrant human tissue kallikrein levels in the stratum corneum and serum of patients with psoriasis: dependence on phenotype, severity and therapy. Br J Dermatol 2007; 156(5):875-83

95. Komatsu N, Saijoh K, Kuk C, et al. Human tissue kallikrein expression in the stratum corneum and serum of atopic dermatitis patients. Exp Dermatol 2007; 16(6):513-19

96. Batra J, O'Mara T, Patnala R, et al. Genetic polymorphisms in the human tissue kallikrein (KLK) locus and their implication in various malignant and non-malignant diseases. Biol Chem 2012;393(12):1365-90

•• **A thorough review covering the vast amount of information available on KLK genetic polymorphisms and their clinical relevance.**

97. Izumi A, Iijima Y, Noguchi H, et al. Genetic variations of human neuropsin gene and psychiatric disorders: polymorphism screening and possible association with bipolar disorder and cognitive functions. Neuropsychopharmacology 2008;33(13): 3237-45

98. Lose F, Batra J, O'Mara T, et al. Common variation in Kallikrein genes KLK5, KLK6, KLK12, and KLK13 and risk of prostate cancer and tumor aggressiveness. Urol Oncol 2013;31(5):635-43

99. Lose F, Srinivasan S, O'Mara T, et al. Genetic association of the KLK4 locus with risk of prostate cancer. PLoS One 2012; 7(9):e44520

100. Nam RK, Zhang WW, Trachtenberg J, et al. Single nucleotide polymorphism of the human kallikrein-2 gene highly correlates with serum human kallikrein-2 levels and in combination enhances prostate cancer detection. J Clin Oncol 2003;21(12): 2312-19

101. Van den Broeck T, Joniau S, Clinckemalie L, et al. The role of single nucleotide polymorphisms in predicting prostate cancer risk and therapeutic decision making. Biomed Res Int 2014;2014:627510

102. Gilgunn S, Conroy PJ, Saldova R, et al. Aberrant PSA glycosylation–a sweet predictor of prostate cancer. Nat Rev Urol 2013;10(2):99-107

103. Kuzmanov U, Smith CR, Batruch I, et al. Separation of kallikrein 6 glycoprotein subpopulations in biological fluids by anion-exchange chromatography coupled to ELISA and identification by mass spectrometry. Proteomics 2012;12(6): 799-809

104. Talieri M, Devetzi M, Scorilas A, et al. Human kallikrein-related peptidase 12 (KLK12) splice variants expression in breast cancer and their clinical impact. Tumour Biol 2012;33(4):1075-84

105. Xu CH, Zhang Y, Yu LK. The diagnostic and prognostic value of serum human kallikrein-related peptidases 11 in non-small cell lung cancer. Tumour Biol 2014. [Epub ahead of print]

106. Jiao X, Lu HJ, Zhai MM, et al. Overexpression of kallikrein gene 10 is a biomarker for predicting poor prognosis in gastric cancer. World J Gastroenterol 2013; 19(48):9425-31

107. Shinoda Y, Kozaki K, Imoto I, et al. Association of KLK5 overexpression with invasiveness of urinary bladder carcinoma cells. Cancer Sci 2007;98(7):1078-86

108. Talieri M, Zoma M, Devetzi M, et al. Kallikrein-related peptidase 6 (KLK6)gene expression in intracranial tumors. Tumour Biol 2012;33(5):1375-83