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

Andreas Scorilas & Konstantinos Mavridis

Department of Biochemistry and Molecular Biology, University of Athens, Panepistimiopolis, Athens 157 01, Greece

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

Andreas Scorilas* and Konstantinos Mavridis
Department of Biochemistry and Molecular Biology, University of Athens, Panepistimiopolis, Athens 157 01, Greece
*Author for correspondence: ascorilas@biol.uoa.gr

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
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
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, 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

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

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

KLK: Kallikrein-related peptidases.
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. 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. A recent study shows that low KLK10 methylation levels are associated with biochemical relapse in prostate cancer patients.

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. 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. Both whole blood and CSF levels of KLK6 were initially reported to be increased in AD patients compared with normal controls. Contrarily, 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. KLK6 CSF and plasma levels are positively correlated with age in normal individuals, and this association is lost or even inverted in AD patients. Decreased KLK6 CSF levels have been proposed as a risk factor for AD development. Interestingly, KLK6 plasma concentrations can be used for the discrimination between AD patients and individuals without neurodegenerative dementia, as well as for calculating the risk for progression of mild cognitive impairment to dementia with vascular component or AD. Furthermore, patients with frontotemporal dementia exhibit decreased KLK6, KLK7 and KLK10 CSF levels compared with control subjects.

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

Ablanter KLK levels are also found in multiple sclerosis (MS). Interestingly, KLK6 participates directly in the pathogenesis of MS by cleaving basic myelin proteins. 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. Patients diagnosed with advanced MS exhibit elevated KLK6 CSF levels compared with neurological controls. 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. 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.

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 keratinocytes, inflammation and ultimately severe skin dysfunction. 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. 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. As far as atopic dermatitis is concerned, serum KLK8 levels are increased, whereas KLK5 and KLK11 levels are decreased; a KLK7 polymorphism (AACC insertion in the 3' UTR) has also been associated with this disease.

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. In the nonmalignant setting, KLK1 SNPs are associated with hypertension, cerebral hemorrhage and cardiovascular disease. A KLK2 SNP is related to male infertility, 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. Prostate cancer has been the cornerstone of KLK-related SNPs research, perhaps due to the importance of PSA.
biopsies panels that include multiple KLK members could also be enhanced by multifactorial biomarker 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.

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.

The significance of several KLK members as individual biomarkers has been corroborated by independent research groups. 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, it is highly probable that the ‘four-kallikrein panel’ will be eventually introduced in clinical practice for prostate cancer.

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 and KLK6 – could help in transforming the envisagement of the incorporation of additional KLKs in future molecular diagnostics into a clinical reality.

Acknowledgements
We apologize to those investigators whose work, due to space constraints, was not cited.

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Predictions for the future of KLKs in molecular diagnostics

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


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


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


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


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

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

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

** A recent study describing the direct clinical applicability of the kallikrein panel.
42. Schmitt M, Sommerhoff CP, Fritz H, et al. Human kallikrein 6 (hK6): a new potential serum biomarker for diagnosis and


