

Hybrid Synthetic Receptors on MOSFET Devices for Detection of Prostate Specific Antigen in Human Plasma

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ABSTRACT: The study reports the use of extended gate field-effect transistors (FET) for the label-free and sensitive detection of prostate cancer (PCa) biomarkers in human plasma. The approach integrates for the first time hybrid synthetic receptors comprising of highly selective aptamer-lined pockets (apta-MIP) with FETs for sensitive detection of prostate specific antigen (PSA) at clinically relevant concentrations. The hybrid synthetic receptors were constructed by immobilising an aptamer-PSA complex on gold and subjecting it to 13 cycles of dopamine electropolymerisation. The polymerisation resulted in the creation of highly selective polymeric cavities that retained the ability to recognize PSA post removal of the protein. The hybrid synthetic receptors were subsequently used in an extended gate FET setup for electrochemical detection of PSA. The sensor was reported to have a limit of detection of 0.1 pg/ml with a linear detection range from 0.1 pg/ml to 1 ng/ml PSA. Detection of 1-10 pg/mL PSA was also achieved in diluted human plasma. The present apta-MIP sensor developed in conjunction with FET devices demonstrates the potential for clinical application of synthetic hybrid receptors for the detection of clinically relevant biomarkers in complex samples.

Field-effect devices are attractive transduction platforms for label-free sensing of bio-recognition events^{1,2}. A typical metal-oxide-semiconductor field-effect transistor (MOSFET) consists of four terminals, namely the gate, drain, source and body. In the majority of applications, including biosensing, the source and body terminals of the device are connected internally, making it a three terminal device². When potential difference is applied at the gate terminal, a conducting channel is created between the drain and source terminals of the device. The electrical charges in the channel can then be made

to flow from the source to the drain by applying a voltage between them, provided the potential at the gate terminal is above the threshold potential of the device. For the purpose of biological and chemical sensing, the gate of the transistor can be extended out of the device to facilitate bio-functionalisation of the gate surface³. The interaction of these extended gates with the analyte of interest changes the effective surface potential of the gate terminal, which in turn modulates the channel conductivity and changes the current between source and drain terminal. The use of extended gates leads to an improved environmental stability of MOSFETs, which allows sensitive detection of biomolecules and provides flexibility in bio-functionalisation of the device. One significant advantage of MOSFETs is their suitability for use in miniaturized measurement systems, thereby allowing ease of integration with electronic readouts. In this regard, the MOSFET system is applicable to a range of point-of-need applications including those within healthcare and environmental monitoring industries⁴. Extended gate FETs have been widely used for DNA hybridisation⁵, aptamer-protein interaction⁶, enzymatic activity detection⁷ and small molecule detection using molecularly imprinted polymers (MIPs)^{8,9}. However, the application of FET devices and in particular MOSFETs for the sensing of proteins using MIPs is yet to be explored.

The process of molecular imprinting involves synthesising synthetic receptors by polymerising functional monomers around a template to form polymer cavities that display steric and chemical selectivity for the template and related molecular species¹⁰. Molecular imprinting offers various advantages over biological receptors in terms of stability, robustness and ease of engineering onto various sensing platforms. The field of molecular imprinting has continuously strived to become a viable alternative to antibodies for detection of proteins; however, conventional imprinting of proteins has proven challenging as a result of their large size, complexity and

dynamic nature¹¹. Epitope imprinting, which uses an epitope presented on the protein surface as the template for imprinting in order to capture the whole protein, has been used as an alternative to whole protein imprinting¹². However, the absence of established crystal structures and known epitopes for most proteins are key barriers for adopting the technique as an alternative to protein recognition and sensing. Hybrid imprinting is an emerging subset of molecular imprinting, which incorporates (bio)receptors with established affinity towards the template into the polymer cavities to generate hybrid synthetic receptors which display superior binding characteristics¹³. Hybrid receptors of this type have been generated using DNA aptamers¹⁴, small molecules¹⁵ and antibodies¹⁶ for the sensitive detection of proteins. We have previously reported a sensitive capacitive sensor for prostate specific antigen (PSA) using hybrid receptors¹³. This study extends our previous findings by reporting a highly sensitive DNA aptamer-based hybrid MOSFET sensor for the detection of PSA. MOSFETs are ubiquitous in all electronic device, can be easily integrated into simple electronics and devices and have been shown to provide better sensitivity than capacitive sensors³. Although the limitations of PSA as a biomarker for PCa have been well noted in the literature, it remains the most commonly used biomarker for screening, monitoring the effectiveness of treatment and assessing likelihood of remission post treatment.¹⁷ Furthermore, the commercial availability of both PSA protein standards and a high affinity anti-PSA aptamer, make it a good target for this first proof-of-concept study.

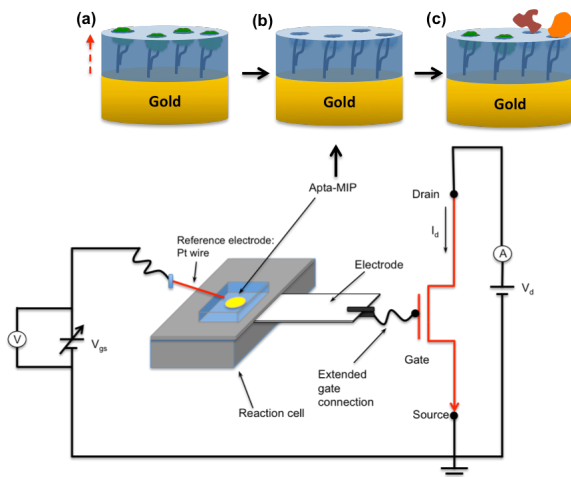


Figure 1: (a) Modification of gold electrodes with anti-PSA aptamer followed by electropolymerisation of polydopamine to imprint the aptamer and PSA. (b) Removal of protein to expose pockets for rebinding. (c) Use of apta-MIP modified electrodes in an extended gate MOSFET setup for rebinding of PSA.

EXPERIMENTAL SECTION

Reagents: Thiol terminated anti-PSA DNA aptamer (5'-HS-(CH₂)₆-TTT TTA ATT AAA GCT CGC CAT CAA ATA GCT TT-3') was obtained from Sigma-Aldrich, UK. PSA was obtained from Merck Chemicals Ltd, UK. Human glandular Kallikrein 2 (hK2) was obtained from RnD Systems, UK. All other reagents were of analytical grade and obtained from Sigma-Aldrich, UK. All aqueous solutions were prepared using 18.2 MΩ cm ultra-pure water from a Milli-Q system with a Pyrogard filter (Millipore, MA, USA).

Apta-MIP preparation: Thiolated aptamer was activated at 95° C for 10 min before being gradually cooled to room temperature for 30 min. Thereafter, 1 μM aptamer in TBST buffer (10 mM Tris-HCl, 10 mM KCl, 10 mM MgCl₂, 0.05% Tween 20, pH 7.4) was incubated with 1 μg/ml of PSA for 1 hour at 37°C. Clean array of gold electrodes (180nm) were then exposed to the resulting aptamer-PSA complex solution for one hour before being rinsed carefully with ultra pure water. The electrodes were subsequently incubated with 1 μg/ml PSA for an additional 30 min before rinsing with ultrapure water and drying under nitrogen. The molecular imprinting step was performed by electropolymerising dopamine on to the aptamer-PSA modified electrode. Briefly, 10 mM PBS buffer (pH 7.4) containing 5 mM dopamine was degassed with nitrogen (10 min) and then electropolymerised using cyclic voltammetry (13 cycles, -0.5 V to 0.5 V vs Ag/AgCl, scan rate of 20 mV/s performed on μAUTOLAB III / FRA2 potentiostat (Metrohm Autolab, The Netherlands). Electrodes were rinsed with water and washed (with stirring) overnight in washing solution (5% v/v acetic acid and 5% w/v sodium dodecyl sulphate (SDS) in water) to remove the PSA template. Electrodes were then rinsed with water to remove residual acid and detergent before being allowed to stabilise in measurement buffer (10 mM PBS (pH 7.4). A non-imprinted 'control' electrode (apta-NIP) was prepared in the same way but in the absence of PSA.

MOSFET measurements: MOSFET measurement were performed on an extended-gate FET sensor consisted of an array of gold electrodes, where MIPs were immobilised and the FET structure, which transduced the binding events on the gold electrode into electrical signals. Arrays of gold (180 nm) electrodes were grown on a glass substrate, using thermal evaporation. The extended gate was fabricated by connecting the Au electrodes, fixed in a reaction cell, to the gate of a n-MOSFET via a metal wire. Detailed information on the specifications of the CMOS chip can be found in literature.¹⁸ To operate the transistor for the measurements, a voltage of 50 mV was applied across the drain to source and gate-source voltage (V_{gs}) was swept from 0 to 4 V. These settings limited the current to less than 75 μA to avoid any changes in V_{gs} due to heating.

Aptamer only evaluation: Experiments with PSA aptamer alone were performed using the same gold evaporated electrodes following the protocol reported by previously.¹⁹ Briefly, electrodes were exposed to thiolated DNA aptamer/6-mercapto- 1-hexanol (MCH, Sigma, UK) immobilization solution for 16 hrs in a humidity chamber. An optimized (1:100) DNA aptamer to total thiol was used to fabricate the biosensor. A high concentration of MCH was prepared in ethanol, which was diluted to working concentration in 10 mM PBS (pH 7.4). Prior to addition of MCH, DNA aptamers were heated to 95 °C for 10 min followed by gradual cooling over 30 min to room temperature. After immobilization, electrodes were rinsed with excess MilliQ water to remove unattached thiols. In order to ensure complete thiol coverage of the gold surface, the electrodes were thereafter backfilled with 1 mM MCH for 1 h. Electrodes were then rinsed with MilliQ water and placed in the 10 mM PBS (pH 7.4) for stabilization.

Sensor Performance: To evaluate sensor performance, electrodes were exposed to 100 μ l of a range of PSA concentrations (10^{-2} – 10^8 pg/ml) in 10 mM PBS buffer pH 7.4. for 30 minutes. Post incubation with PSA the electrodes were washed thoroughly with PBS. For plasma experiments, blood obtained from a healthy female volunteer was spun down (3200 rpm, 20 minutes) to remove the red blood cells. The resultant plasma was diluted 1000 times in a PBS buffer of pH 7.4 and then spiked with varying concentrations of PSA (1, 5, 10 pg/ml PSA). For detection of binding, a voltage of 50 mV was applied between the drain and source (V_d or V_{ds}) to operate the transistor and the gate-source voltage (V_{gs}) was swept from 0.0 to 5.0 V. All electrical measurements were conducted using an Agilent B1500A semiconductor device analyser. Measurements for all experiments were recorded for 4 different devices.

RESULTS AND DISCUSSION

Fig. 2(a) shows the response of the device upon incubation with increasing concentrations of PSA in PBS. Fig. 2(b) shows the transfer characteristics of the device upon binding to different concentrations of PSA. An initial change of 30 mV was obtained for lower concentrations (1-10 fg/mL) following which a linear response was obtained from 0.1 to 10 pg/mL of PSA. Above 100 pg/mL, the voltage shifts remain almost constant, suggesting saturation of the binding sites. A maximum voltage change of 158 ± 43 mV was observed at a concentration of 1 μ g/mL of PSA. The apta-NIP experiments revealed voltage shifts of 20 times lower (8 ± 3 mV, Fig. 2) than that observed in the apta-MIP for the same concentrations of PSA. The initial change of 30 mV at concentrations between 1 and 10 fg/mL could be attributed to small amounts of weak binding of PSA to the aptamer causing a conformational change and hence changes in the distribution of charges in the bilayer. Beyond 0.1

pg/mL a case can be made for specific interaction of the protein with the apta-MIP surface, increasing the shift in voltage as a result of the aptamer charges being screened and the protein binding to the polymeric cavity dominating the charge behaviour at the surface. The FET sensor can detect local net-charges of PSA because of the smaller sensing depth (the Debye length) compared to the size of protein. It is hypothesised that upon rebinding of PSA (with a certain net charge) to the apta-MIP, there is a change in the electrochemical double layer and in the charge distribution of the bilayer, which causes a variation in the potential drop at the gate of the MOSFETs. A positive shift in voltage observed upon binding of PSA to apta-MIP corresponds to an increase in negative charges on the surface of the sensor; it is hypothesised that this is an effect of net negative charge of PSA.²⁰ The apta-MIP bio-FET device was approximately 10 times more sensitive than an impedance spectroscopy (EIS) sensor reported previously by our groups¹⁴ suggesting that the MOSFET device is a better platform for biosensing with apta-MIPs. In the case of the apta-NIPs, the absence of a polymeric cavity restricts much of the binding to PSA, which leads to a small change in voltage. It is proposed that the aptamers become entrapped within the polymeric network in the absence of PSA and hence, post-polymerisation access to the aptamer-binding site is severely hindered.

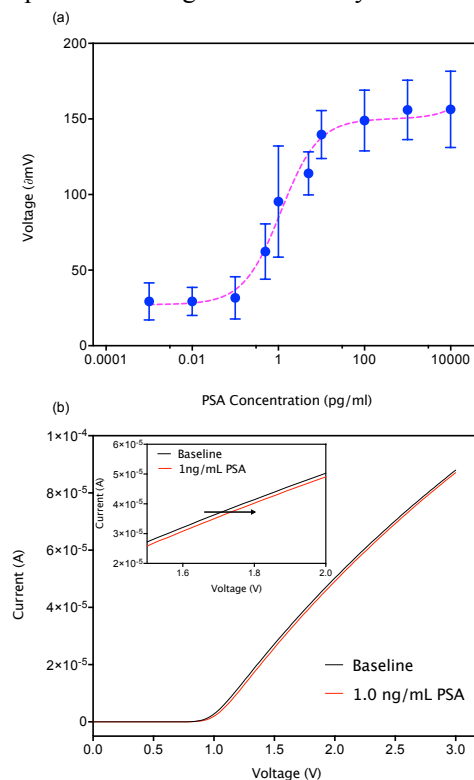


Figure 2: (a): Dose response of PSA with apta-MIP FET device. (b) Transfer characteristics curve of apta-MIP FET device binding to PSA.

To understand the selectivity of the sensor, the apta-MIP bio-FET device was subjected to stringent controls; comparing the response with the protein hK2, which has 80% homology with PSA, in a non-competitive assay. The response to 1 $\mu\text{g}/\text{mL}$ hK2 was significantly lower (56 ± 14 mV) than the change observed with an equivalent concentration of PSA (158mV) (Fig. 3). This suggests that whilst some cross-reactivity is observed the system is able to distinguish between the template and closely related proteins. Interestingly in the previous study¹⁴, where binding to aptamer alone was investigated, lower cross reactivity with hK2 (18%) was observed when compared to the apta-MIP (28%). This suggests that the enhanced cross-reactivity observed with the apta-MIP arises as a result of interactions between hK2 and the polymer network. The aptamer itself has been designed to limit cross-reactivity with structurally related proteins and as such targets one relatively small epitopic region on the PSA protein. It is not surprising therefore, that when we build a polymer network around the aptamer-PSA complex, which introduces a second layer of interactions that likely occur over a much larger surface area of the protein than that of the aptamer, that a greater degree of cross-reactivity with a highly homologous protein is observed. When challenged with human serum albumin, a structurally unrelated protein, minimal response was observed. It should also be noted that the concentration hK2 in clinical samples is 100 times lower than PSA and therefore in practice it is unlikely to interfere in binding of PSA.²¹

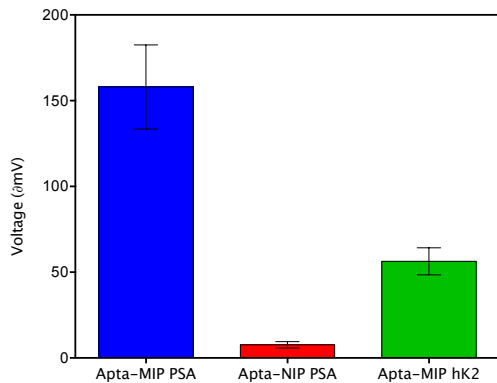


Figure 3: The Apta-MIP sensor demonstrated a significantly larger response to 1 $\mu\text{g}/\text{mL}$ PSA when compared to the Apta-NIP system. Some cross-reactivity was observed when the apta-MIP was challenged with 1 $\mu\text{g}/\text{mL}$ hK2.

It was important to compare the apta-MIP sensor with an aptamer only device so as to understand whether an improvement in recognition performance was achieved as a result of imprinting. On comparing an apta-MIP with an aptamer alone FET sensor (see curve for aptamer/MCH in Fig. 4), a significantly lower sensitivity was observed for the aptamer only sensor. The aptamer only FET displayed a shift of 18 mV compared to 158 mV shift of apta-MIP at 1 ng/mL PSA. A negative shift

in voltage was observed at 0.1 $\mu\text{g}/\text{mL}$, which could be a result of non-specific binding of PSA to the MCH self-assembled monolayer. A positive shift in voltage was observed upon increasing the concentration, however the response was much lower than for the apta-MIP sensor, suggesting an improvement in the sensitivity of the hybrid MIP system. A previous study has showed that varying the polymer thickness influences sensor performance, which supports the hypothesis that the polymer plays a role in PSA recognition.¹⁴

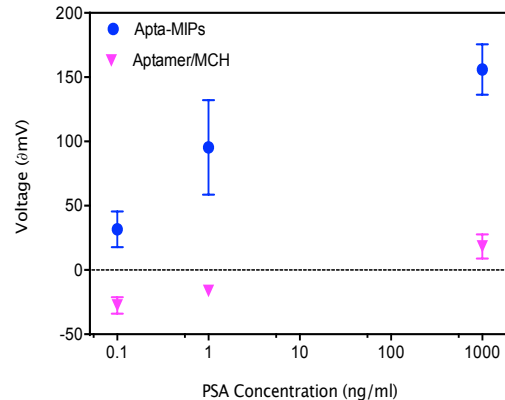


Figure 4: Comparison of apta-MIP and aptamer/MCH response to PSA. The aptamer only system displays poorer sensitivity than the apta-MIP system.

The sensor was further tested in human plasma to evaluate its potential use in clinical applications. Human plasma from a healthy female volunteer was used to ensure no interference from any background PSA levels. Spiking the plasma with PSA allowed for the measurement of known levels of PSA in the plasma (low background PSA) whilst maintaining the ratio of PSA/plasma proteins. The high sensitivity of this device for PSA demonstrated *in-vitro* means that clinically relevant levels of PSA (4-10 ng/mL²²) can be readily detected even following a 1/1000 dilution of plasma. When 1/1000 diluted female plasma (no PSA added) was applied to the sensor little change in response was observed (Fig. 5). This suggests good resistance of the apta-MIP surface to plasma protein fouling. Upon incubation of the sensor with plasma containing 1-10 $\mu\text{g}/\text{mL}$ PSA (spiked after dilution of plasma) a linear response was observed. Although the sensitivity was lower than observed with simple buffer PSA solutions, the results are highly encouraging given the ability to directly detect clinically relevant concentrations. The loss in sensitivity is likely explained by protein-protein interactions, which has also been observed in aptamer ELISA assays²³. The free PSA used in the current assay is derived from seminal fluid, which is known to interact with complement proteins, anti-chymotrypsin and albumin present in plasma, which could prevent it from interacting with the apta-MIP hence, lowering the sensitivity of the sensor. Free PSA

present in patient blood however is internally cleaved, thereby avoiding interaction with anti-chymotrypsin²² and so an improvement in system sensitivity maybe observed with real clinical samples.

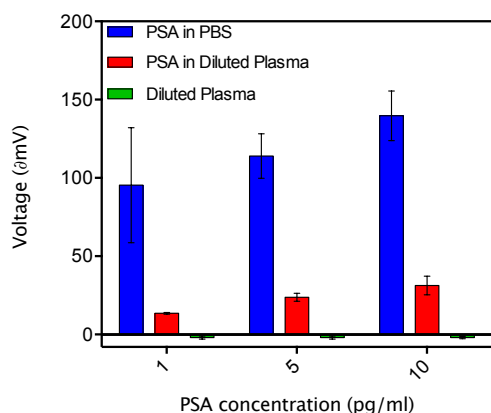


Figure 5: Apta-MIP response in 1000X diluted plasma spiked with PSA.

CONCLUSIONS

In conclusion, the work presents for the first time the use of MOSFET devices for sensing a disease related protein using a hybrid synthetic receptor. A sensitive MOSFET apta-MIP device has been developed that has improved sensitivity when compared with an equivalent aptamer only MOSFET sensor and a previously reported impedimetric apta-MIP device. In addition, negligible response of the apta-MIP surface to diluted plasma and the systems intrinsic high sensitivity meant that PSA could be detected in 1/1000 diluted plasma over a clinically relevant concentration range. The combination of hybrid MIP receptors with MOSFET devices could help develop the next generation of robust hybrid biosensors that could be translated to other clinical markers.

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The author declares no competing financial interest.

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REFERENCES

1. A. Poghossian and M. J. Schöning, *Electroanalysis*, 2014, **26**, 1197–1213.
2. P. Estrela and P. Migliorato, *J. Mater. Chem.*, 2007, **17**, 219–224.
3. P. Estrela, D. Paul, Q. Song, L. K. J. Stadler, L. Wang, E. Huq, J. J. Davis, P. K. Ferrigno and P. Migliorato, *Anal. Chem.*, 2010, **82**, 3531–3536.
4. C.S. Lee, S.K. Kim, M. Kim, *Sensors* **2009**, *9*, 7111–7131.
5. F. Uslu, S. Ingebrandt, D. Mayer, S. Böcker-Meffert, M. Oden-thal and A. Offenhäusser, *Biosens. Bioelectron.*, 2004, **19**, 1723–1731.
6. K. Maehashi, T. Katsura, K. Kerman, Y. Takamura, K. Matsu-moto and E. Tamiya, *Anal. Chem.*, 2007, **79**, 782–787.
7. Senillou, N. Jaffrezic-Renault, C. Martelet and S. Cosnier, *Talanta*, 1999, **50**, 219–226.
8. Z. Iskierko, M. Sosnowska, P.S. Sharma, T. Benincori, F. D'Souza, I. Kaminska, K. Fronc and K. Noworyta, *Biosens. Bi-oelectron.*, 2015, **74**, pp.526–533.
9. M. Lahav, A. B. Kharitonov, O. Katz, T. Kunitake, I. Willner, *Anal. Chem.*, 2001, **73** (3), 720–723
10. K. Haupt and K. Mosbach, *Chem. Rev.*, 2000, **100**, 2495–2504.
11. N. W. Turner, C. W. Jeans, K. R. Brain, C. J. Allender, V. Hla-dy and D. W. Britt, *Biotechnol. Prog.*, 2006, **22**, 1474–1489.
12. H. Nishino, C.-S. Huang and K. J. Shea, *Angew. Chem. Int. Ed.*, 2006, **45**, 2392–2396.
13. W. Bai and D. A. Spivak, *Angew. Chem.*, 2014, **126**, 2127–2130.
14. P. Jolly, V. Tamboli, R. L. Harniman, P. Estrela, C. J. Allender and J. L. Bowen, *Biosens. Bioelectron.*, 2016, **75**, 188–195.
15. D. Dechtrirat, N. Gajovic-Eichelmann, F. F. Bier and F. W. Scheller, *Adv. Funct. Mater.*, 2014, **24**, 2233–2239.
16. T. Miyata, M. Jige, T. Nakaminami and T. Urugami, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 1190–1193.
17. J.H. Hayes, M. J. Barry, *J. Am. Med. Assoc.*, 2014, **311**, 1143–1149.
18. N Formisano, N. Bhalla, M. Heeran, J. R. Martinez, A. Sarkar, M. Laabei, P. Jolly et al. *Biosens. Bioelectron.*, 2016, **85**, 103–109.
19. P. Jolly, N. Formisano, J. Tkáč, P. Kasák, C. G. Frost and P. Estrela, *Sens. Actuators B*, 2015, **209**, 306–312.
20. W. M. Zhang, J. Leinonen, N. Kalkkinen, B. Dowell and U. H. Stenman, *Clin. Chem.*, 1995, **41**, 1567–1573.
21. Klee, G. G.; Goodmanson, M. K.; Jacobsen, S. J.; Young, C. Y.; Finlay, J. A.; Rittenhouse, H. G.; Wolfert, R. L.; Tindall, D. *J. Clin. Chem.* 1999, **45**, 800–806.
22. N. Savory, K. Abe, K. Sode and K. Ikebukuro, *Biosens. Bio-lectron.*, 2010, **26**, 1386–1391.
23. S. P. Balk, Y.-J. Ko and G. J. Bublely, *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.*, 2003, **21**, 383–391.

GRAPHICAL ABSTRACT:

