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Circulating cell-free DNA: Translating prostate cancer genomics into clinical care

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A B S T R A C T

Only in the past decade tremendous advances have been made in understanding prostate cancer genomics and consequently in applying new treatment strategies. As options regarding treatments are increasing so are the challenges in selecting the right treatment option for each patient and not the least, understanding the optimal time-point and sequence of applying available treatments. Critically, without reliable methods that enable sequential monitoring of evolving genotypes in individual patients, we will never reach effective personalised driven treatment approaches. This review focuses on the clinical implications of prostate cancer genomics and the potential of cfDNA in facilitating treatment management.

1. Prostate cancer

Prostate cancer (PC) is the most commonly diagnosed cancer and the second leading cause of cancer related death in men. Prostate specific antigen (PSA) is detected in the blood from men and levels are increased both in benign prostatic hyperplasia (BPH) and due to tumour development in the prostate. Increased PSA levels are thus followed up by further examinations and pathological evaluation of tissue biopsies from the prostate to confirm presence of cancer lesions. As many men today test their PSA, a vast majority of men are diagnosed with localized and less advanced, often indolent, tumours (Moore et al., 2009).

Early PC detection is the best chance of cure, and elevated PSA levels in the blood may indicate presence of PC prior to any symptoms. PSA screening is therefore an attractive strategy in preventing mortality but a large proportion of men that are diagnosed with PC will never develop from an indolent to a lethal disease within their lifetime (Schröder et al., 2014). The test thus leads to vast overtreatment of men, and still men with aggressive PC are missed. Having surgical or radiological treatment of the prostate is frequently followed by poorer quality of life and many men unnecessarily suffer these consequences due to overdiagnoses (Loeb et al., 2014). There is thus a need of strategies to further stratify patients at risk and spare those with indolent tumours from unnecessary biopsies and radical treatments. Although there is an increasing number of available commercial diagnostic tests based on molecular markers, their benefit on survival and cost-effectiveness are yet not properly evaluated in head-to-head comparisons (Carlsson and Roobol, 2017; Cucchiara et al., 2018).

In men who are diagnosed with metastatic disease or who progress after their initial treatment there is still no cure. Androgen deprivation treatment (ADT) is a potent treatment for metastatic PC (mPC) and has until 2004 been the only life-prolonging systemic treatment for mPC (Nuhn et al., 2019). Although most tumours respond to ADT, and in some men the response lasts for several years, some men have primary resistance to treatment and invariably all men develop secondary resistance and progress to castration-resistant prostate cancer (CRPC).

In recent years approved treatment options prolonging survival for men with mCRPC have significantly increased, mainly with taxane chemotherapy (Johann Sebastian De Bono et al., 2010; de Leeuw et al., 2015; Petrylak et al., 2004) and second-generation androgen inhibiting agents abiraterone and enzalutamide (Johann S. De Bono et al., 2011; Scher et al., 2012). Furthermore, novel treatment options are being investigated with additional androgen targeting, and strategies to inhibit other pathways as mono- or combinational therapies.

PSA is used as a marker of disease recurrence and subsequently informs on treatment-response in patients. Neuroendocrine tumours are an aggressive subset of PC that do not secrete PSA (J. M. Mosquera et al., 2013). Critically, PSA has limitations as a biomarker in this setting, and can furthermore not guide on treatment selection.

Conclusively, there is an unmet need for biomarkers in early disease for better stratifying patients with aggressive tumours from those not in need of further intervention.

In advanced stages biomarkers are needed for early detection of

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recurrence, treatment selection, tracking treatment response/early detection of treatment resistance.

Real-time monitoring could enable fast adaptation of treatment strategies in order to cumulatively prolong survival and quality of life in patients with PC.

2. Molecular characterization of prostate cancer

2.1. Primary prostate cancer

PC is a multifocal, molecularly heterogeneous disease (Abeshouse et al., 2015; Baca et al., 2013; Cooper et al., 2015; Lindberg et al., 2013) so that each tumour can be composed of many different tumours, all with individually different potential to grow and progress. Extensive integrative molecular characterization of PC has been made possible by the technical developments in next-generation sequencing (NGS) which has substantially increased our understanding of PC biology.

In primary PC ETS transcription factor fusions and rearrangements are the most frequently occurring aberrations, with fusion between the promotor of gene *TMPRSS2* and *ERG* being detected in approximately 50% of patients (Gasi et al., 2011; Hermans et al., 2009; Tomlins et al., 2005). *TMPRSS2* is a prostate specific and androgen regulated gene and located 3 Mbp from *ERG* and the fusion leads to overexpression of a truncated ERG protein. It is thought to be one of the earliest molecular alterations, detected already in prostate intraepithelial neoplasia (PIN), the precursor stage of PC (Cerveira et al., 2006; DeMarzo et al., 2003; J.-M. Mosquera et al., 2008). Copy number variations (CNVs) with chromosome 8p24 gain (including *MYC*) and 8q21 losses (including *NKX3-1*) are other frequently occurring aberrations in primary tumours as well as focal CNVs like *PTEN, TP53* and *RB1* loss. The mutation rate in primary prostate is relatively low compared to other tumour types with *SPOP* mutations being the most frequent; occurring in 6–15% of tumours (Barbieri et al., 2012; Taylor et al., 2010). Molecular characterization of primary PC by The Cancer Genome Atlas Research Network (TCGA) could categorize 74% of primary cancers studied into seven distinct subtypes (n = 333) with *SPOP* mutations detected exclusively in ETS-fusion negative samples. Possible clinically actionable aberrations in AR, DNA-repair, PIK3 and MAPK signalling pathways were variably affected across all defined subtypes although *SPOP* and *FOXA1* mutated patients where associated with the highest AR transcriptional activity (Abeshouse et al., 2015) in this cohort.

The clinical relevance of the different subtypes in primary PC is complex and still subject for investigation. Additional aberrations to *TMPRSS2-ERG* fusion seem necessary for cancer onset and data on the predictive value of the fusion are conflicting (Gasi Tandefelt et al., 2014). Some events like 3p13 loss (including genes *FOXP1, RYBP, SHQ1*) is associated with ERG + subset and tumours harbouring both aberrations manifest a more aggressive molecular subtype (Krohn et al., 2013). Furthermore, prognostic gene expression signatures are identified within ERG + subtype (Gasi Tandefelt et al., 2013; Kamoun et al., 2018) across several independent cohorts.

Copy number burden has also been shown to better predict poorer progression (Hieronymus et al., 2014) than PSA or Gleason grade and a recent study found that whole chromosome-arm aneuploidy in primary tumours could identify patients that where 5-fold more likely to progress to lethal disease (Stopsack et al., 2019). Prospective data and comparison between novel tests are necessary to evaluate their benefit. In order to acquire relevant data, these tests need to be cost-efficient and easy to use in the clinical setting.

2.2. Tumour evolution and metastatic prostate cancer

Tumours progression can be described as a trajectory following Darwinian evolution (Greaves and Maley, 2012) where cancer cell-clones adapt to selective pressures that are applied through changed metabolic rate, new environment, treatments etc. Tumours genomic composition is thus changed over time and may require a different treatment strategy from the one initially selected.

Although primary tumours are multifocal and heterogeneous, Liu *et al.* showed by characterizing copy number patterns from multiple metastasis in each individual that metastasis are of monoclonal origin (Liu et al., 2009) and acquire sub-clonal events throughout progression. More recent data supports these findings and additionally shows that convergent evolution occurs under selective pressure of ADT where several metastases develop androgen-receptor (AR) aberrations independently (Bova et al., 2016; Grasso et al., 2012; Gundem et al., 2015; Kumar et al., 2016) over time.

In a study by Haffner *et al.*, identified the focus responsible for the lethal metastatic spread in one patient and demonstrated that the origin was not the high-grade bulk tumour but a small, lower grade focus in the prostate, strengthening the relevance of investigating the molecular background of tumour tissue in addition to pathological evaluation (Haffner et al., 2013).

After the initial metastatic lesion, the metastatic spread likely follows seed-to-soil with more molecular similarities between metastasis that are in close proximity than to the primary tumour they originated from (Gundem et al., 2015).

Comprehensive molecular characterization of PC metastasis shows many similarities to primary disease, regarding pathways that are affected, but also some important differences. One difference seen is an increased mutation rate from approximately 1 mutation/Mb (Abeshouse et al., 2015) to 4.4 mutations/Mb (D. Robinson et al., 2015).

Most striking difference is the emergence of AR CN gains and mutations in over 60% of patients which are absent in primary tumours. Enrichment of aberrations in *TP53, PTEN,* and PI3K/AKT pathway is observed as well as increased numbers of low-frequent (occurring in less that 3%) recurrent mutations (Armenia et al., 2018).

An interesting finding was that also the germline samples that where sequenced from PC patients had an enrichment of mutations in the DNA-repair pathway (Pritchard et al., 2016).

Across all exome sequencing reports, overlapping data is emerging of recurrent events driving PC, as well as a combination of less frequent drivers unique to each individual that require large datasets to detect. Merging previously published data with novel whole-genome sequences (WGS, n = 930) revealed 22 novel aberrations as well as non-coding mutations in *FOXA1* and *NEAT1* acting as drivers in PC (Wedge et al., 2018). Recently an amplification was identified by WGS in an enhancer 620 kB upstream of *AR* resulting in AR transcriptional activation in 81% of mCRPC tumours. This finding suggests that up to 85% of mCRPC have de-regulated AR-pathway by duplications or mutations (D. A. Quigley et al., 2018), a finding supported by recent data (De Laere et al., 2019).

Neuroendocrine PC is a subset of tumours that do not express AR or PSA, lead to visceral metastasis, and associate with particularly poor clinical outcome. Adenocarcinomas can, as a mechanism of treatment resistance to androgen deprivation and AR targeting treatments, acquire neuroendocrine features. The genetic characteristics of this aggressive subset of tumours are amplification of *MYCN* and losses of *RB1* and *TP53* function (Beltran et al., 2011; Ku et al., 2017; Puca et al., 2019).

3. Cell-free DNA and clinical implications of PC genomics

3.1. Cell-free DNA in prostate cancer

Low pass whole-genome and targeted deep sequencing can detect circulating tumour DNA (ctDNA) in men with metastatic PC (mPC) but not in localized disease (Hennigan et al., 2019) thus the utility of cfDNA in early prostate cancer is currently limited. Recent cfDNA studies show differences in nucleosome footprints (Snyder et al., 2016; Ulz et al., 2019) between healthy and tumour cells which may expand the use of

cfDNA to early detection.

In metastatic PC up to 90% of metastasis are spread to the bone making tissue sampling difficult and, in many cases, not possible. DNA sequencing studies with contemporarily collected biopsies from metastasis and plasma have high overlap between detected aberrations in circulating cell-free (cfDNA) and tissue (Frenel et al., 2015; Wyatt et al., 2017), demonstrating that cfDNA is a surrogate source of tumour material. Aberrations can be missed in cfDNA due to low levels of tumour DNA fraction in some samples, but can also provide additional information that is missed in tissue due to under-sampling (Vandekerkhove et al., 2019).

Different tumour clones that are present in one patient are represented in the circulation and can be tracked over time (Carreira et al., 2014; Frenel et al., 2015). Serial collection of cfDNA can therefore be used as a tool to study the biology behind cancer progression and emerging treatment resistance mechanisms. Another potential of ctDNA is to facilitate the translation of prostate cancer genomics into clinically practical multi-purpose biomarkers.

3.2. Clinical implications of cancer genomics

3.2.1. AR-pathway

In addition to ADT that is initially effective in most men, several studies have now shown that the more effective androgen targeting treatments like abiraterone and enzalutamide, and systemic treatments like docetaxel significantly prolong symptom-free disease and overall survival when applied in combination with ADT in men with hormone-sensitive metastatic disease (Fizazi et al., 2019; N. D. James et al., 2017; Nicholas D. James et al., 2016; van Soest and de Wit, 2015).

AR aberrations are not detected in primary tumours or hormone-naïve metastasis but appear in tumours after ADT with AR CN gain in 30% (Visakorpi et al., 1995) and point mutations in approximately 20% (D. Robinson et al., 2015; Taplin et al., 1999) of cases. The most common mutations are T878A and H875Y but the ligand-binding domain (LDB) of AR is a hot-spot for additional mutations. Progesterone and cortisol stimulated L702H (Van De Wijngaart et al., 2010) have particularly high activation by cortisol as double mutant with T878A (Matias et al., 2002), two mutations often co-occurring at progression to abiraterone in cfDNA studies (Carreira et al., 2014; Romanel et al., 2015; Wyatt et al., 2016). In patients who become resistant to abiraterone a “steroid switch” to the glucocorticoid dexamethasone from prednisolone leads to continued response (Lorente et al., 2014) in 40% of cases. The mechanism behind this is not known but one possibility could be that particular point mutations (or combination thereof) could inform on those men who might benefit from a “steroid switch” (Zhao et al., 2000).

Additional recurrent AR mutations are the bicalutamide-induced W742 C/L (Carreira et al., 2014), F877L that is associated with enzalutamide treated patients (Wyatt et al., 2016) and the less characterized V716M (Culig et al., 1993), among others (Shi et al., 2002). W742C/L, H875Y, F877L and T878A act as agonists instead of antagonists to androgen deprivation treatments but the mutations in L702H, V716M, H875Y and T878A also result in promiscuous receptors that can be activated by other ligands like oestradiol, progesterone or hydrocortisone (Shi et al., 2002). Functional studies of AR mutant clones that emerge at resistance to novel antiandrogens such as F877L and double mutant F877L/T878A demonstrate that these clones are sensitive to previously used antiandrogen bicalutamide highlighting possibilities to prolong successful anti-androgen treatment for subgroups of patients (Lallous et al., 2016).

The androgen receptor expresses several splice variants (AR-Vs) both in normal prostate and in cancer tissue (Watson et al., 2015). AR-V7, a constitutively active splice variant, has been correlated with primary resistance to abiraterone and enzalutamide treatment when detected in circulating tumour cells (CTC's) (Antonarakis et al., 2014). Numbers of CTC's as well as levels of cfDNA in the circulation (as marker of tumour burden) have been shown to predict response to treatments (Johann S. De Bono et al., 2008; De Laere, Oeyen, et al., 2019; Mehra et al., 2018; Torquato et al., 2019) and several studies show that AR aberrations associate with treatment resistance to abiraterone and enzalutamide (Conteduca et al., 2017; Romanel et al., 2015; Wyatt et al., 2016).

The largest sequencing effort of mCRPC tumour tissue to date (n = 444) investigated the clinical outcome of several common genetic aberrations in abiraterone and enzalutamide treated patients (Abida et al., 2019). AR point mutations and CN gain associated to treatment resistance in concordance to previous reports, but did not associate with overall survival. Although the expression of AR-V7 and other splice variants increased with treatment to both taxanes and androgen targeting drugs, the expression did not associate with treatment resistance or overall survival in this cohort.

3.2.2. DNA damage repair genes

DNA damage repair (DDR) aberrations occur in 10–19% (Abeshouse et al., 2015; Armenia et al., 2018) of primary cancers and in up to 25% of castration-resistant PC (CRPC) (Armenia et al., 2018; D. Robinson et al., 2015). In recent studies that by whole-exome sequencing, characterized germline samples collected from men with metastatic PC the frequency of DDR mutations was increased from that in general population (3%), to ~8–12% (Pritchard et al., 2016). The increase in prevalence suggests that carriers might be at higher risk to develop metastatic disease and, if so, DDR gene sequencing could in the future select patients for a more aggressive treatment regime early.

Poly (ADP-ribose) polymerase (*PARP*) inhibitors hinder the release of DNA repair protein PARP1. In combination with DDR impaired tumour cells this inhibition induces programmed cell death (Hopkins et al., 2015). In a phase II study (TOPARP), the *PARP* inhibitor olaparib was evaluated for responses in a cohort of fifty patients that had previously received taxane treatment and 98% had also received enzalutamide or abiraterone prior to enrolment. In patients that harboured defects in *BRCA2/1, ATM, PALB2* and other DDR genes (n = 16) response to treatment was reached in 88% of cases (Mateo et al., 2015). During this trial sequential plasma was collected and the follow-up study elegantly demonstrates the potential of cfDNA as a multi-purpose biomarker (Goodall et al., 2017). It demonstrates concordance between detected aberrations in circulation and simultaneously collected tissue, strengthening the value of screening cfDNA in order to identify patients with DDR germline and somatic aberrations. Subclones with genomic changes that restored DDR function emerged at progression in cfDNA samples identifying mechanisms of resistance that were not detected in tissue (Goodall et al., 2017; D. Quigley et al., 2017).

Platinum-based chemotherapy cross-links DNA, stalling the replication fork (Lord and Ashworth, 2016) and is an effective treatment in DDR defected ovarian and breast cancers. Individual cases have been reported with remarkable responses to cis-platin (Beltran et al., 2015) and carboplatin in prostate cancer patients, all harbouring different DNA-repair deficiencies (Zafeiriou et al., 2019).

Approximately 2–5% of mCRPC patients have mismatch repair (MMR) or micro-satellite instability (MSI) with mutations in *MSH2* and *MSH6* (Pritchard et al., 2014; D. Robinson et al., 2015). Although small percentage of patients, this could be an opportunity for many individuals to gain a treatment option with Pembrolizumab, a PD-1 inhibitor that is approved for use in any tumour type with MMR defects (Le et al., 2017).

CDK12 mutated tumours are present in 3–7% of mCRPC (D. R. Robinson et al., 2017; Wu et al., 2018) and have a distinct immunophenotype making them another promising candidate for immunotherapy. Clinical implications of *CDK12* were investigated in a cohort that combined tissue and cfDNA sequencing data from three different centres (Melissa A. Reimers et al., 2019). *CDK12* aberrated patients had shorter time to metastasis and CRPC suggestive of a more

aggressive subtype but did not reach significance as an independent variable in multivariable analysis. mCRPC patients stratified by biallelic loss of *CDK12* will be enrolled in the IMPACT trial, NCT03570619 (Melissa Andrea Reimers et al., 2019), to assess the response rate to the combination of immunotherapies nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4).

3.2.3. PI3K pathway

PTEN loss and deregulation of other *PI3K* pathway genes (*PIK3CA/B, AKT, PIK3RI*) are significantly higher in mCRPC (~49%) than primary tumours (below 20%) pointing toward activation of *PI3K* pathway having an important role in tumour progression (Abeshouse et al., 2015; Abida et al., 2019; D. Robinson et al., 2015). *PTEN* loss are the most common aberration and is essential for tumour development in ETS positive tumours in *in vivo* studies and in aggressive subtypes of PCs (Ahearn et al., 2016; Ku et al., 2017; Zong et al., 2009; Zou et al., 2017). A strategy in combating *PI3K* driven neoplasm is inhibiting *AKT*, the junction of *PI3K* signalling pathway. Recent understanding of the crosstalk between *PI3K* inhibition and *AR* signalling suggests that a combination of androgen and *AKT* inhibitors could be a successful strategy for a subset of patients (Carver et al., 2011; Schwartz et al., 2015). Trials investigating the combination for mCRPC are undergoing and will determine possible association with *PTEN* loss and effect of treatment (NCT02525068), and the benefit of *AKT* inhibition in *a priori* selected patients with *AKT* mutations (NCT03310541).

4. Discussion and future perspectives

In recent years there has been a tremendous increase of data on PC genetics. There is a high level of consistency in the reports on recurrently found aberrations in PC (Abida et al., 2019; Armenia et al., 2018; Chen et al., 2019; D. R. Robinson et al., 2017) but yet novel discoveries are made through integrative efforts that are combining several independent cohorts in order to increase study power (Wedge et al., 2018). The increased understanding of PC biology and evolution of the disease have consequently led to opportunities to develop new treatments against novel promising targets, of re-purposing already existing treatments and of gaining survival benefit by administrating treatments earlier in the disease pathway.

While exploratory research is adding to our understanding and increasing treatment targets and treatment options (Adams et al., 2019), feasible and fast means of mapping the background of each patient to enable the correct treatment strategy at the right time are necessary. Novel technical developments have enabled detection of tumour DNA in plasma cfDNA and provide a powerful tool in tracking changes over time. Importantly, clinical relevance of the underlying genetics needs to be elucidated as outcomes for patients with mCRPC are still dismal and novel treatment strategies urgently needed.

Circulating cell-free DNA and other liquid biopsies have enabled tracking of different emerging genetic aberrations present in the tumour in “real-time”. Despite that cfDNA only provides complementary information to tissue and other liquid biopsies (reviewed in (Heitzer et al., 2017)) there is growing evidence for the biomarker utility of this minimally invasive source of tumour material.

Mechanisms of resistance have been tracked and better understood due to the possibility of cfDNA sequential tumour sampling (Annala et al., 2017; Carreira et al., 2014; De Laere et al., 2019; Frenel et al., 2015; Lallous et al., 2016; Mayrhofer et al., 2018; Romanel et al., 2015) and several described studies have leveraged cfDNA to identify potentially clinically valuable biomarkers (Table 1). The results of these biomarker studies are inconsistent and there is yet not a single study that prospectively validates identified markers. Possible explanations to this inconsistency are; the use of different sequencing platforms and assays, applying different thresholds for calling variants and missing important co-occurring events due to use of relatively small targeted panels in most studies.

Table 1
Clinical evaluation of genomic biomarkers in mCRPC patients by cfDNA.

Study reference	Treatment	Evaluated target genes	Analysis method	Cohort size	Key clinical implications
Romanel A et al. 2015.	Abiraterone	*CYP17A1, AR*	Ion Torrent targeted panel	Patients: 97 Samples: 247	Shorter PFS and OS with aberrant *AR* (gain/mutations).
Azad, 2015.	Abiraterone, Enzalutamide	Exon 8 of *AR*	Target seq. Roche 454, XLR70 kit; aCGH	Patients: 62 Samples: 62	Shorter PFS with aberrant *AR*
Wyatt A et al. 2016.	Enzalutamide	*AR, ASXL1, BRCA1/2, CHD1, CHEK2, CTNNB1, FOXA1, HSDB31, KDM6A, MED12, KMT2A, MYC, OR5A1, PIK3CA, PTEN, SCN11A, SPOP*, and *TP53*	Ion Torrent targeted panel; aCGH; deep sequencing of AR	Patients: 65 Samples: 119	*RB1* loss and *MET* gain patients have significantly shorter PFS.
Annala et al. (2018)	Abiraterone, enzalutamide	73-gene panel incl.; *AR, ATM, BRCA2, CHEK2, MED12, MLH1, MSH2/6, PTEN, RB1, TP53*	NimbleGen SeqCap EZ Choice target capture	Patients: 202 Samples: 201	Deleterious alterations in *BRCA2, ATM* and/or *TP53* TTP.
Belic, 2018	Abiraterone, enzalutamide	*AKAP9, APC, AR, ATM, BRAF, BRCA1/2, CTNNB1, EGFR, ERG, FOXA1, GNAS, GRIN2A, HRAS, KRAS, TP53, MED12, MLL, MLL2/3, MLLT3, NOTCH1, NRG1, RB1, PIK3CA/B, PIK3R1, PTEN, SPOP, TMPRSS2, ZFHX3*	Targeted and WGS by TruSeq Nano and Capture kits respectively	Patients: 125 Samples: 334	No significant associations with treatment response reported.
Conteduca, 2017	Abiraterone, enzalutamide	*AR*	ddPCR	Patients: 265 Samples: 265	*AR* gain associates with PFS and OS.
De Laere et al. (2019)	Abiraterone, Enzalutamide	*AR, TP53*	Targeted and WGS with ThruPLEX DNA-seq ki	Patients: 168 Samples: 252	*TP53* outperforms *AR* aberrations in predicting outcome.
Goodall J et al. 2017	Olaparib	113 gene panel incl.; ATM, BRCA1, BRCA2, CDK12, CHECK2, FANCA, PALB2	Targeted and WES with Agilent Sure SelectXT v6 and Qiagen Mix-n-Match V2 respectively	Patients: 49 Samples: 254	BRCA2 and PALB2 reversion mutations emerging at resistance
Quigley D et al. 2017	Olaparib, Talazoparib	73-gene panel incl.; *AR, ATM, BRCA2, CDK12, PTEN, RB1, TP53*	NimbleGen SeqCap target capture	Patients: 2 Samples: 4	BRCA2 reversion mutations emerging at resistance.
Mehra et al. (2018)	Docetaxel, cabazitaxel	-	Quant-IT Picogreen HS DNA quantification kit	Patients: 571 Samples:2502	cfDNA independent prognostic marker for rPFS and OS

Methodological advances in DNA sequencing (Filges et al., 2019; Mansukhani et al., 2018) and finding better ways of identifying the tumour fraction in cfDNA samples (Annala et al., 2018) can further improve the sensitivity to detect timely changes in the future.

In breast cancer tracking individual mutations could predict recurrence after curative treatment as early as 8 months prior to clinical recurrence. This prediction was superior when tracking events identified in sequential cfDNA samples over those aberrations identified in primary tumour tissue (Garcia-Murillas et al., 2015). Recently, the same group showed the utility of ctDNA tracking to predict relapse in early breast cancer (Garcia-Murillas et al., 2019) on average 10.7 months prior to clinical criteria. An initial WGS and whole-exome sequencing (WES) study on cfDNA in early prostate cancer failed to detect ctDNA in early PC (Hennigan et al., 2019) but other targeted approaches that enable higher sequencing depth might be better suitable for this purpose. More data is needed to investigate the potential of relapse detection with ctDNA in prostate cancer.

Combination of drugs based on individual genome data and computational predictive models is an approach to successfully keep treatment resistance at bay in HIV (Bock and Lengauer, 2012). This strategy could be explored for managing treatment resistance in cancer.

Computational models that predict mechanisms of resistance in cancer, identify that ten resistant subclones are present in any radiologically detectable lesion (Bozic and Nowak, 2014) confirming the difficulty of single targeting treatment strategies. Tracking emerging subclones in sequentially collected cfDNA confirmed these findings (Bozic et al., 2016) and point toward the need of expanding treatment choices and investigating tolerable treatment combinations. Treatment strategies that take advantage of PC evolution trajectories could better keep tumour growth at bay with decreased treatment doses (Enriquez-Navas et al., 2016; Gatenby et al., 2019) in order to enable treatment combinations.

Furthermore, computational models implementing PC genomics data is accelerating drug discovery for PC by predicting potentially successful re-purposing of already approved drugs and identifying promising novel drug targets (Wedge et al., 2018; Workman et al., 2019), steadily increasing treatment options.

Even though the data on genetic characterization and its clinical relevance is encouraging, none of the genetic markers are yet prospectively validated and consistency in the definition of clinically relevant genetic markers are lacking.

In summary, the ability to track genomic changes over time by cfDNA opens tremendous opportunities in learning about PC vulnerabilities that could be exploited for patient benefit but analytical standardization and prospective clinical trials are urgently warranted.

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