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Target Acquired: Progress and Promise of Targeted Therapeutics in the Treatment of Prostate Cancer

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Abstract: Cancer is fundamentally a genomic disease caused by mutations or rearrangements in the DNA or epigenetic machinery of a patient. An emerging field in cancer treatment targets key aberrations arising from the mutational landscape of an individual patient's disease rather than employing a cancer-wide cytotoxic therapy approach. In prostate cancer in particular, where there is an observed variation in response to standard treatments between patients with disease of a similar pathological stage and grade, mutation- directed treatment may grow to be a viable tool for clinicians to tailor more effective treatments. This review will describe a number of mutations across multiple forms of cancer that have been successfully antagonised by targeted therapeutics including their identification, the development of targeted compounds to combat them and the development of resistance to these therapies. This review will continue to examine these same mutations in the treatment and management of prostate cancer; the prevalence of targetable mutations in prostate cancer, recent clinical trials of targeted-agents and the potential or limitations for their use.

Keywords: Precision medicine, prostate cancer, targeted therapeutics.

CANCER: A GENOMIC DISEASE

Cancer is fundamentally a genomic disease. While cancer development can be triggered and aggravated by a number of factors, all human cancers are the result of changes in the DNA of a subject or in their epigenetic regulatory machinery. These aberrations can be inherent and hereditary [1, 2], precipitated by viral infections or other disease [3], a result of exposure to carcinogenic agents [4], or simply spontaneously arising in somatic cells. Each of these mechanisms has the potential to be exploited as molecular targets for therapeutic agents. This review will address the current ability to examine genomic information from individual patients' cancers and how this can impact the unique landscape of prostate cancer at the clinical level. This review will continue by examining the rise and use of modern targeted therapeutic agents across cancer treatment and potential uses of these in the treatment of prostate cancer at various stages of the disease.

EXPLORING THE HUMAN GENOME

The first draft sequence of the human genome published in 2001 [5, 6], provided the foundation for clinical genome sequencing across a multitude of diseases. The initial public effort, using revolutionary but limited technology, produced a 3-gigabyte DNA sequence at a cost of $3 billion (US) in an undertaking that took thirteen years to complete. Today, the Next Generation Sequencing (NGS) technologies allow for a whole genome to be sequenced thirty fold for a little over $1,000, in a matter of days, providing feasibility for the application of genome sequencing in medical practice [7]. These technical advances also allow for high throughput targeted and exome sequencing, transcriptome sequencing and more recently methylome sequencing of bisulphite treated DNA, making it possible to examine both the genetic and epigenetic drivers of tumourigenesis. It has now become the detailed and integrated analysis of this data rather than the collection itself that limits the applications of genomic sequencing. Dr. Elaine Mardis of The Genome Institute, Washington, a leader in the field of genomics, commented that on analysing whole genome sequencing data "the required expertise to 'solve' each case included molecular and computational biologists, geneticists, pathologists and physicians with exquisite knowledge of the disease and of treatment modalities, research nurses, genetic counsellors, and IT and systems support specialists, among others" causing analysis to cost upwards of $100,000 [8]. However, ongoing advances in computational technologies and computational analyses are continuing to aid the interpretation of sequencing data and their routine clinical implementation. The efficient analysis of sequencing data is, of course, a necessary tool for any prospective mutation-directed treatment regimen.

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TARGETED THERAPIES: VISION OR REALITY OR BOTH?

Vision

Mutation-directed treatment is an emerging medical field that aims to use an individual patient's germline or tumour genetic profile to guide clinical decisions. Mutation-directed treatment aims to identify predictive and prognostic genetic markers which can be used to predict disease outcome and provide patient specific tailored treatment regimens [9]. In oncology in particular, current chemotherapy, radiation and surgical intervention strategies can result in significant short-term toxicities and long-term functional side effects. By accurately predicting response to specific targeted therapeutics it is possible to minimise the impact and associated health care costs of these side effects while simultaneously maximising the efficacy of treatment.

Reality

Producing targeted therapeutics has been an aspiration of both scientists and clinicians for many years. In 1902 the first link between genetic inheritance and predisposition to a disease was made by Sir Archibald Garrod in his work on alkaptonuria [10] and then in 1956 the first example for gene-dependent selective toxicity was discovered for the anti-malarial drug primaquine [11]. However it is only relatively recently that non-hormonal targeted therapies have become a reality in the treatment of cancer. Malignancies including melanoma, breast, lung, and gastric cancers, and leukaemia are no longer treated solely by tissue type but are now able to be treated based on their molecular subtype, discoveries which will be covered in further detail in this review (Table **1**).

An additional challenge for mutation-directed treatment is the development of companion diagnostics to identify patients for treatment with targeted therapeutics. An excellent example of dual development of diagnostic and therapeutic is HercepTest and Herceptin (trastuzumab) which were approved for use only six months apart in 1998, the former to assess levels of HER2 expression and the latter treat HER2-positive breast cancer. Among other agencies, the US Food and Drug Administration (FDA) has recognised the importance for accurate diagnostics to accompany targeted therapeutics releasing guidelines in 2011 stating their intent to assess drugs and their companion diagnostics simultaneously [12]. Despite the progress in other tumour types, the question remains; is mutation-directed treatment a viable approach to the treatment of prostate cancer?

Table 1. Clinical Trials of Targeted Therapeutics in Prostate Cancer.

TARGET	*EGFR*	*EGFR*	*HER2*	*PI3K/Akt/mTOR Signalling Pathway*	*ALK*	*BCR-Abl*	*BRACA1/2*
CANCERS APPROVED FOR TREATMENT		*KRAS-dependent*		mTOR	*EML4/ALK*		
	NSCLC, CRC, Breast Cancer, Leukaemia	CRC	Breast Cancer	Her2-negative Breast Cancer, Renal Cell Carcinoma	NSCLC	Chronic Myeloid Leukaemia	Breast Cancer
THERAPEUTIC CLASS OF AGENT	Erlotinib	Cetuximab	Trastuzumab	Rapalogs	Crizotinib	Dasatinib	Olaparib
	Tyrosine Kinase Inhibitor	Chimeric IgG1 monoclonal antibody	Humanised IgG1 monoclonal antibody	Rapamycin derived mTORC1 inhibitors	Alk targeting small molecule inhibitor	Tyrosine Kinase Inhibitor	PARP Inhibitor
CaP TRIALS {*n*} denotes prostate cancer patients in multi-cancer trial	Phase II Clinical Trial: n=29 (chemotherapy naïve)[a]	Phase II Clinical Trial: n=38[b]	Phase II Clinical Trial: n=18 (CRPC)[c]	Pilot Study Trial n=13[d]	No trial to date	Phase III, randomised double-blind with docetaxel: n=1522 chemotherapy naïve mCRPC[e]	Phase II Clinical Trial: n=60{3}[f]
CLINICAL RESPONSES	2 patients had a partial response: 31% had clinical benefit: 9% progression free at 12 months	34% progression free survival rate at end point. Dependent on WT *KRAS*	Poor efficacy	Clinical response in 17%: warrants further study		No comparable difference to docetaxel	One patient with *BRCA2* mutation – had >50% reduction in PSA levels, and decreases in bone metastases

[a][113], [b][60], [c][114], [d][70], [e][102], [f][107].

THE PROMISE OF MUTATION-DIRECTED TREATMENT IN PROSTATE CANCER

The treatment of prostate cancer in the economically developed countries with long life expectancies is a significant medical challenge. Prostate cancer has the highest incidence rate, yet has on average a comparatively high 5 year survival rate of 92% [13]. Currently, determining the subset of patients with an aggressive form from those with a more indolent form of this disease remains problematic, as there are few biomarkers which have been identified to estimate the severity of prostate cancer progression.

In routine clinical practice, patients are stratified according to the risk of disease progression based upon serum Prostate Specific Antigen (PSA), digital rectal examination, the Gleason Score of a diagnostic biopsy, and a variety of other factors such as age, race, family history, and comorbidities formulated through one of a number of nomagrams. Together, these inform clinicians and patients on which course of treatment to pursue – be it active surveillance, hormone therapy, radiation, chemotherapy, or a radical prostatectomy. These prognostic and diagnostic tools however, simply enable the clinician to estimate the patient's disease severity at that point in time – they give no indication of if, how or when the cancer will progress. While low-risk prostate cancer patients have a high likelihood of disease-free survival regardless of treatment chosen [14], men presenting with high-risk disease are at greater risk for treatment failure by single treatment modalities [15]. This inability to accurately predict the outcome of a person's disease is a prevalent cause of the high-rate of overtreatment currently seen in prostate cancer patients, and this can often result in significant morbidity as the side effects of currently available therapies can be severe.

These factors alone would seem to point to prostate cancer treatment being an ideal field for mutation-directed treatment decision making and targeted therapeutics, however further unique aspects of the biology of prostate cancer, such as multifocality and heterogeneity, add both exciting avenues of enquiry and frustrating complications to this pursuit.

Inter-tumour Heterogeneity in Prostate Cancer

Prostate cancer patients have been observed to vary widely in their response to similar treatment regimens. Broad differences are elicited in all clinical outcomes following treatment including length of progression-free-survival, time to bio-chemical relapse, time to castrate resistance following androgen-deprivation therapy, time to mortality or even complete response [16, 17]. This degree of variation in response to current clinical practices is in part explained by a high degree of genetic differences between prostate cancers in different men [18]. In contrast to cancers such as leukaemia and non-small cell lung cancer (NSCLC) where the same driver mutation occurs in a high frequency of patients [19], prostate cancer in different men is often caused by genomic alterations (mutations, chromosomal rearrangements or epigenetic events) in different genes in different pathways, mutations that react differently to various treatments [20, 21]. It is this environment to which targeted therapeutics are well suited. By determining the drivers of an individual man's cancer it is possible to predict to which treatments he will respond. However, it is into this space that the other facet of prostate cancer heterogeneity intrudes and it is an aspect which puts considerable pressure on the potential efficacy of targeted therapies.

Intra-tumour Heterogeneity in Prostate Cancer

Many cancers are thought to originate from a single progenitor cell which harbours multiple cancer causing mutations and from which all subsequent cancerous cells arise, linked by specific germline mutations [22]. However there is growing evidence that some forms of cancer can have multiple distinct origins in the same tumour [23, 24]. This polyclonal origin is believed to be the norm rather than the exception in prostate cancer with approximately 80% of prostate cancers being shown to have more than one genetically distinct sub-population [25, 26]. Moreover, prostate cancer heterogeneity is amplified by significant genetic instability over the decade-spanning natural history of the disease. In a mechanism termed chromoplexy, multiple double stranded DNA breaks accrue at specific sites. These events cause a high prevalence of genomic recombination events, which can have a profound impact on cancer progression [27]. Several studies have reported that the multi-focal development of prostate cancer can be due to both; the spontaneous origin of cancer at different sites in the prostate, and the spread of existing cancer which is then given its own characteristics by widespread genetic instability [28, 29]. Some studies have taken this idea even further to suggest that all focal sites in a prostate cancer are in fact of monoclonal origin but the impact of these studies has been hampered by small sample size [30]. When a prostate cancer spreads to other organs however, it has been shown that most metastatic sites within a patient originate from the same clone in the primary tumour [31]. These findings have also indicated that metastases that spread to bone or lymph nodes are more likely to have similar genetic characteristics [32].

Assessing Heterogeneity

That within any particular example of prostate cancer several genetically distinct cancerous sites are present poses a particular challenge to mutation-directed treatment. There is the possibility that in identifying and actioning a targetable mutation a previously dormant clone that is unsusceptible to this treatment can be triggered to develop into a more aggressive disease by over-treatment [17]. As such it remains imperative to the use of targeted therapies to be able to accurately analyse multiple sites within the prostate for targetable mutations, an issue that is not yet resolved and yet an issue that is similarly faced when deriving the Gleason Score for prostate cancer, which remains one of prostate cancer's primary prognostic tools.

The changing landscape of cancer treatment has already afforded a number of ways to more efficiently treat specific molecular subsets of cancer across a number of cancer types. With this in mind this review will now address examples of successes in the development and use of targeted therapeutics and the possibilities or impracticalities of

translating these drugs for use in improving prostate cancer diagnostics, prognostics and treatment.

KEY GENETIC MUTATIONS THAT HAVE BEEN THERAPEUTICALLY TARGETED IN CANCER

EGFR Family Kinases

EGFR as a Therapeutic Target

Mutations of the epidermal growth factor receptor (EGFR) are often referred to as tumourigenesis "driver mutations". EGFR-activating mutations are frequently seen to be early events in cancer development and members of the EGFR (HER) family of tyrosine kinases regulate a number of cellular processes including; cell proliferation, apoptosis, neo-vascularisation, and cell motility [33, 34]. 10 – 20% of patients with NSCLC at an advanced stage are positive for EGFR activating mutations and this has proven to be predictive in response to various receptor tyrosine kinase inhibitors (TKIs) in particular erlotinib and gefitinib [34]. In prostate cancer, one study found that 13% of primary prostate tumours had mutations in *EGFR* and 36% had EGFR over-expression. Prostate cancer patients with overexpression of EGFR are also known to have a significantly increased risk of biochemical relapse [35]. A different study, observing Chinese men, found 10% of those profiled had an activating mutation in *EGFR* [36]. A thorough investigation of EGFR in 71 castrate-resistant prostate cancers showed there was EGFR expression in over 75% of castrate-resistant cancers. This expression was not seen to be homogeneous within the cancer however, with most EGFR-expressing cancers showing 10-75% of tumour cells with EGFR expression [37]. In a trial of gefitinib by the National Cancer Institute of Canada-Clinical Trials Group in 40 patients with hormone-refractory prostate cancer, no PSA or objective measurable response to treatment was recorded and quality-of-life decreased [38]. A suggested reason for this ineffectiveness of EGFR blockade therapies in prostate cancer is the subsequent autocrine activation of alternative receptors by the over-expression of the HER receptors and ligands. This cascade up-regulates HER2 and HER3 ultimately leading to increased HER3 phosphorylation which bypasses EGFR to activate the downstream PI3K/Akt survival pathway [39]. This potential mechanism is supported by pre-clinical data that has shown that erlotinib is effective in prostate cancer cells where EGFR levels were significantly higher than levels of the HER2 oncogene [40]. This leads to the possibility that combined inhibition of EGFR along with HER2 or HER3 may be effective in treating prostate cancer, a possibility supported in part by pre-clinical data finding that dual inhibition of EGFR and HER2 sensitised hormone-naive prostate cancer cells to androgen deprivation therapy [41].

HER2 as a Therapeutic Target

A total of 15 – 30% of breast cancers have been shown to have amplification and/or overexpression of the *HER2/neu* oncogene and as such will respond to treatment with trastuzumab, a humanised IgG1 monoclonal antibody against the HER2/neu protein [42]. Resistance to trastuzumab has predictably been observed in pre-clinical experiments in which there are alterations downstream of HER2 in the HER2-signalling pathway, for example *PI3K* mutation, or down regulation of PTEN or p27 [43]. Additionally a number of studies have so far investigated targeting HER2 in the treatment of prostate cancer. The role of HER2 in prostate cancer, both in a treatment and prognostic sense remain unclear. A recent review of HER2/neu serum profiling has shown that the HER2 extracellular domain is detectable in the serum of prostate cancer patients and is mainly over-expressed in aggressive disease and castrate- resistant cancers [44] while a murine study of human xenografts showed that adding trastuzumab to standard chemotherapy could delay the onset of hormone- independence [45]. HER2 over-expression is seen in five out of eight prostate tumour metastases when compared to matched primary tumours and co-expression of EGFR and HER2 has been identified in four out of eight metastases in the same cohort [46], additionally a single agent clinical trial of lapatinib, a dual EGFR and HER2 inhibitor, found a response in a small subset of un-profiled patients with castrate-resistant disease [47]. Two phase II studies of the use of the HER2 inhibitor pertuzumab in castrate-resistant prostate cancer failed to show any objective result of a PSA decline of greater than 50%. The results of these studies can perhaps be attributed to the prevalence of PTEN loss in prostate cancer or conversely to the fact that patients' tumours in these studies were not profiled for HER2 or not able to be profiled for HER2 overexpression [48, 49], an oversight that is shared with many other studies of targeted therapies in prostate cancer.

KRAS as an Indicator of Resistance

A formative event in the development of several cancer types is the mutation of the *KRAS* oncogene, which instead of being a targetable event in itself can offer resistance to EGFR-targeting therapies. KRAS mutation is especially prevalent in colorectal cancer (CRC) occurring in approximately 40% of patients. During the past decade, the introduction of targeted therapies and novel chemotherapeutic agents has increased the median overall survival rate of patients with metastatic CRC [50, 51]. One drug, cetuximab, a chimeric IgG1 monoclonal antibody which binds with high specificity to EGFR and has been found to be effective in patients who have a wild type KRAS [52]. However mutant KRAS has been associated with resistance to TKI therapies, and a poor prognosis with cetuximab (Fig. **1**) [53, 54]. Metastatic progression as well as disease relapse in prostate cancer has been linked to the activation of EGFR [55]. A recent phase II clinical trial involving thirty-eight patients with metastatic castration resistant prostate cancer that had previously failed docetaxel single-agent therapy, showed a 34% incidence of progression-free survival at a 12 week time-point after further treatment with docetaxel in combination cetuximab [56].

Another recent clinical study involving eighty-eight Chinese patients with prostate cancer has shown that *EGFR* mutations are more common than *KRAS* mutations in prostate cancer (with *KRAS* mutations occurring in ~2.5% of patients) [36]. This study also indicated that *EGFR* and *KRAS* mutations may be mutually exclusive in prostate cancer and therefore anti-EGFR treatment may be useful in

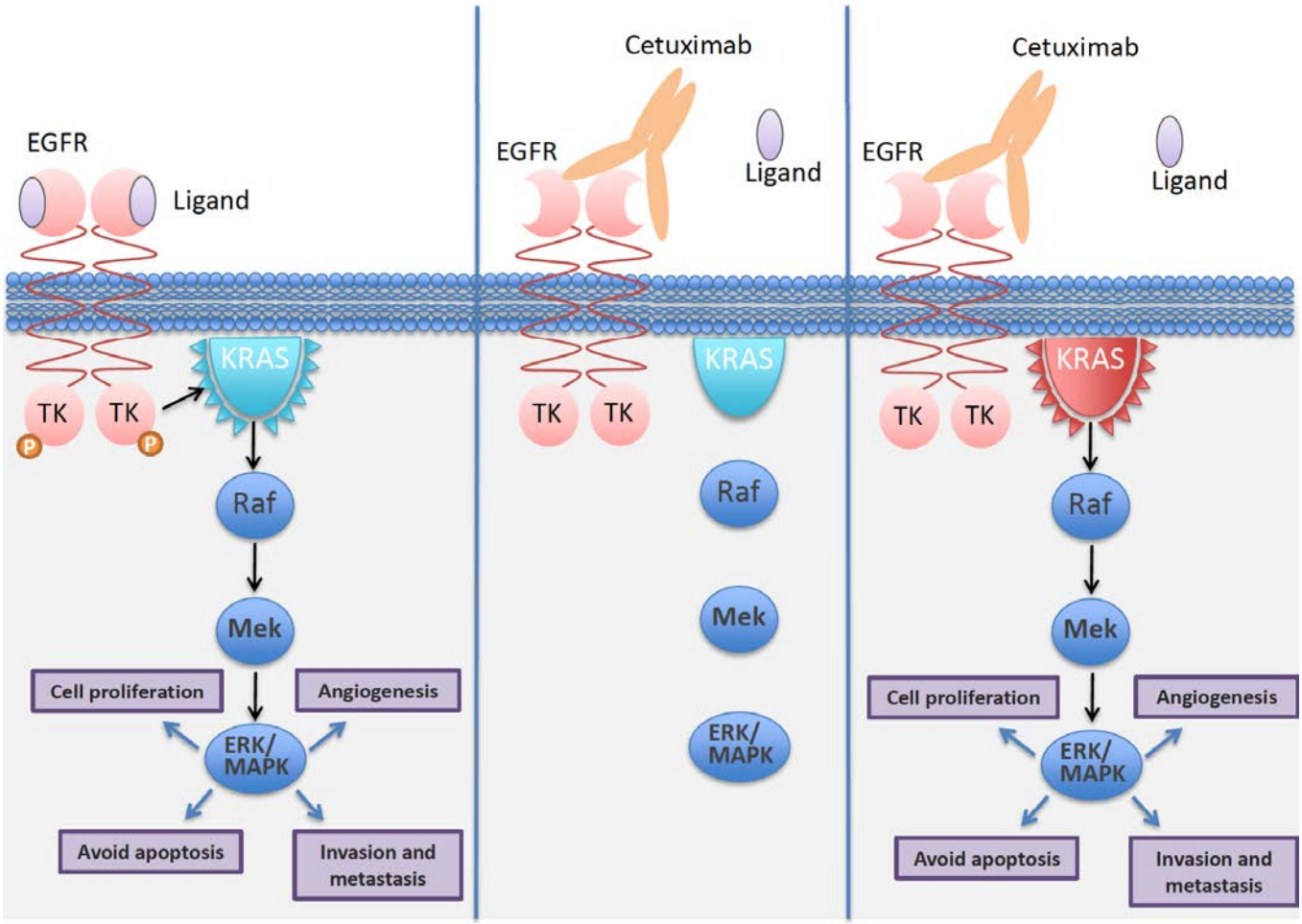

Fig. (1). The action of cetuximab. In many cancers, including colorectal cancers (CRC), increased activity of the EGFR tyrosine kinase (TK) leads to over activation of the MAPK/ERK signalling cascade through the GTPase KRAS (A). Cetuximab, a chimeric IgG1 monoclonal antibody currently used in CRC, is able to bind to EGFR in place of its activating ligand and inhibit the activation of these oncogenic pathways (B). However in approximately 40% of CRC patients, an activating mutation in KRAS is able to bypass EGFR inhibition and activate the downstream cascades, making treatment with cetuximab ineffective (C).

treating prostate cancer. WT KRAS status is now used as a predictive tool in determining treatment with anti- EGFR inhibitors in patients with CRC and this is currently being clinically tested in prostate cancer where the mutational status of each gene may prove to be as important as in CRC.

PI3K/Akt/mTOR Pathway

PI3K/Akt/mTOR as a Therapeutic Target

The phosphatidylinositol 3-kinase/protein kinase-B/mammalian target of rapamycin (PI3K/Akt/mTOR) signalling pathway has been shown to be activated in cancer more frequently than any other pathway [57, 58]. The primary effect of PI3K pathway alterations is the downstream activation of mTOR in its two complexes, mTORC1 and mTORC2 to stimulate angiogenesis, protein synthesis, proliferation, survival and metastasis in many cancers including CRC and renal cell carcinoma [59, 60]. So far, despite its importance in cancer progression and metastasis, few PI3K-targeted agents have progressed through clinical trials due to lack of selectivity and toxic events [61]. Two therapeutic agents derived from rapamycin, everolimus and temsirolimus, inhibit the mTORC1 complex and have been approved by the FDA for use limited to four specific cancer sub-types. The recently completed BOLERO-3 study showed that addition of everolimus to trastuzumab and vinorelbine treatment resulted in a significant increase to progression-free survival in trastuzumab-resistant metastatic breast cancer patients [62]. Second-line everolimus treatment proved effective in metastatic renal cell carcinoma resulting in 19% of patients experiencing a partial response and 62% of patients achieving a stable disease with median progression-free survival of 8 months [63]. 43% of primary prostate cancers and 65% of metastatic disease present with alterations to the PI3K pathway. The majority of these alterations in prostate cancers are deletions or mutations in *PTEN* (see below) but also common are deletions of *FOXO1* and *FOXO3* and amplification of *PIK3CA* [64]. In addition pre-clinical studies have shown that exposure to long-term androgen deprivation therapy and docetaxel increased signalling in the PI3K pathway through phosphorylated Akt

possibly contributing to castrate-resistance [65]. Despite promising preclinical results, the inhibition of mTORC1 with everolimus and temsirolimus in castrate-resistant prostate cancer has been unsuccessful with few responses and short time to clinical progression [66]. In addition pharmacodynamics has shown in men with intermediate- to high-risk localised prostate cancer that rapamycin and its derivatives are clearly inhibiting mTORC1 action but with no significant outcomes in pathological or clinical response [67, 68]. This inaction is possibly because of rapalogs' inability to inhibit mTORC2 which is known to activate Akt in prostate cancer cells [69]. Prostate cancer treatment may benefit more from treatment with a dual mTORC1/2 inhibitor but at this time these agents are only just beginning to enter early stage clinical trials. However recent pre- clinical studies on castrate-resistant prostate cancer cell lines trialling a novel dual PI3K and mTORC1/2 inhibitor NVP- BEZ235 has found promising results for future prostate cancer treatment. Cells treated with BEZ235 were found to have significant reduction in the levels of active Akt protein and were sensitised to docetaxel overcoming docetaxel resistance [70]. In a similar study with docetaxel-resistant castrate-resistant cell lines BEZ235 was also able to sensitise the cells to treatment with a TKI, showing a synergistic effect and another study showed that BEZ235 improved the response of prostate cancer cells to radiotherapy [71, 72]. A further study of prostate cancer cell lines determined that the action of BEZ235 was independent of *PTEN* mutational status inducing cell death in both *PTEN* wild-type and *PTEN* null cells indicating that *PTEN* deficiency should not affect treatment outcomes [73]. The dual inhibition of mTORC1/2 appears at this early stage to be an exciting avenue for future prostate cancer treatment, however it will be necessary to determine the likelihood and prevalence of toxic side-effects that have outweighed the benefits of previously developed PI3K pathway inhibitors [61].

PTEN as a Prognostic Marker

Loss of function of the PTEN tumour suppressor through a variety of mechanisms has been observed in many types of human tumour; including brain, kidney, endometrium, breast, and prostate at a frequency that can rival p53 silencing [74]. In prostate alone, decreases in PTEN protein levels are observed in 70% of surgically removed cancers [75]. The prognosis of prostate cancer patients with PTEN loss is statistically poorer, with loss of expression being correlated with advanced stage, high grade cancer [76], promotion of microvessel growth [77], a shorter time to metastasis [78], and an increased incidence of biochemical and local recurrence [79]. Furthermore, studies have discovered that combined deletion of PTEN and p53 leads to the development of much more aggressive tumours when compared to cancers with deletions in one gene [80]. Notably, PTEN expression for prognostic purposes in prostate cancer must be profiled at the protein level as it has been demonstrated that PTEN can be antagonised at the protein level without changes to DNA or RNA expression [81]. However with the correlations between PTEN expression and severity of prostate cancer, an early-stage PTEN screen would be a valuable resource in informing treatment decisions.

Targetable Fusion Mutations in Cancer

ALK as a Therapeutic Target

The *EML4-ALK* fusion oncogene is a mutant kinase that has been shown to exhibit strong oncogenic activity but can be effectively suppressed by ALK-targeting small molecule inhibitors [82]. *EML4-ALK* appears in 3-6% of NSCLC patients or 10-20% when the cohort is restricted to younger, non-smoking patients but a phase III trial of the ALK- inhibitor crizotinib in combination with platinum chemotherapy in this subset of patients found a 51% reduction in the risk of disease progression and a more than three-fold increase in the rate of response to radiotherapy [83]. Resistance to crizotinib in cancer can be conferred by two secondary mutations in the EML4-ALK kinase domain, [84]. Both mutations act by reducing the binding affinity of crizotinib for ALK and consequently another ALK inhibitor may not be affected in the same manner [85, 86]. Different ALK fusion mutations have also been reported in a growing group of other cancers including lymphoma, renal and soft tissue but to date NSCLC is the only malignancy to benefit from ALK-targeting. ALK activation has recently been identified in different metastatic sites in five different patients with castration-resistant prostate cancer using phosphoproteomic profiling [87] however no trials of ALK- inhibitors have been conducted in prostate cancer treatment at the time of writing, leaving this a possible avenue of further enquiry.

BCR-ABL as a Therapeutic Target

The *BCR-ABL* fusion gene produces a continuously active tyrosine kinase lacking the regulatory elements typically found in tyrosine kinases [88-90]. The constitutive kinase activity of BCR-ABL is well documented in chronic myeloid leukaemia (CML) [91] and can be treated by imatinib, an effective BCR-ABL inhibitor [92, 93]. Imatinib was demonstrated in clinical trials to increase the survival of CML patients past 8 years to 95.2%, similar to the rate of survival in the general population, essentially curing the disease. Less than 1% of patients in the study died of leukaemia progression [94]. Regardless of imatinib's success in treating CML, a number of resistance mechanisms have developed including; methylation of *SOCS3* which activates STAT3 to increase cell proliferation [95], the release of reactive oxygen species from the mitochondria elevating levels of 8-oxo-guanine [96], increased expression of BCR- ABL, the expression of multidrug-resistant P-glycoprotein (MDR-1), or mutation of the BCR-ABL kinase domain [97]. Additionally the effects of imatinib have had limited translational capabilities in different cancers. The effects of imatinib have been negligible in prostate cancer across multiple trials testing imatinib against PSA-progression following local therapy, biochemical recurrences, and castrate resistant disease [98-100]. An imatinib second- generation drug, dasatinib, showed promising effects in phase II clinical trials in hormone-refractory prostate cancer with observations that dasatinib has a positive response in bony metastases [101]. Although, further phase III trials have shown no survival benefit when compared to docetaxel in metastatic castrate-resistant disease [102]. However, there have been few trials which have directly profiled BCR-ABL

in early-stage or castrate-resistant prostate cancer and as such, success with BCR-ABL inhibitors was always unlikely. Future trials of imatinib or its derivatives must first establish the presence of BCR-ABL or a similar target in prostate cancers before attempting treatment.

BRCA-1 and BRCA-2 as a Therapeutic Target

A significant breakthrough in mutation-directed treatment was the identification of the correlation of *BRCA-1* and *BRCA-2* mutations to the risk of breast cancer in women. There is a 45-65% occurrence of women carrying a mutation in either of these genes developing breast cancer by the age of seventy [1]. This increased cancer risk is not only restricted to breast cancer, as these mutations also increase the risk of ovarian, colon and prostate cancers [103]. In particular men with a *BRCA-1/2* mutation have a 1.6-8.6 fold greater risk of developing prostate cancer [104]. Specifically for men who possess a *BRCA-1* mutation, studies have shown carriers develop prostate cancer at a younger age and with a more aggressive form of disease, distant metastases, and an increased mortality rate as compared to non-carriers [2, 105]. Furthermore a germline mutation in *BRCA-2* has been found to be present in approximately two per cent of men who present with prostate cancer at an early age [106]. Although this mutation could be used as a prognostic it should be used in conjunction with additional genetic screening since it only affects around 3% of prostate cancers and may be of more use in young men to inform them of their need for regular screening later in life. BRCA deficient cells have become a target of mutation-directed treatment as they have been found to exhibit synthetic lethality, a situation in which disruption of one of two alternate pathways promotes survival while the subsequent inactivation of the second pathway with a therapeutic drug will cause cell death. In this case, BRCA deficient cells lack the homologous- recombination DNA repair mechanism but retain the mechanisms for the alternative base-excision DNA repair allowing tumour cells to escape the apoptotic cell death normally triggered by DNA damage. A recent phase I clinical trial of 60 patients, 22 of which had a germline *BRCA-1* or *BCRA-2* mutation has shown that *BRCA* mutation carriers with solid tumours, including prostate, had a higher response rate to olaparib, a Poly ADP-ribose Polymerase (PARP) inhibitor [107]. All patients in this trial had failed standard therapies to their disease, and five per cent of those recruited had prostate cancer. One patient with castration- resistant prostate cancer was a *BRCA-2* mutation carrier, and following olaparib treatment, had more than a 50% reduction in PSA level and resolution of bone metastases. In total, 63% of the patients in this trial who reached the end stage of treatment had a clinical benefit from this PARP-inhibitor. Olaparib enables selective cytotoxicity by preventing base- excision repair occurring in BRCA deficient cells, but normal cells that have functional homologous recombination pathways are not affected. Despite the benefits of olaparib as a cancer therapy, resistance to this therapy can occur. Therapy resistance arises through an intragenic in-frame deletion that removes the initial *BRCA-2* mutation and restores BRCA-2 protein function in tumour cells [108]. This deletion that restores BRCA-2 function is facilitated by error-prone DNA repair, originally caused by the loss of BRCA-2 [109]. A recent study which sequenced samples from 45 patients with either localised prostate cancer, metastatic hormone naïve prostate cancer, or metastatic castration resistant prostate cancer found that a *BRCA-2* gene deletion occurred frequently in castrate resistant prostate cancer (20%) [110]. Therefore PARP inhibitors could possibly be an avenue of treatment in castrate-resistant disease, however further studies are required to determine their effectiveness in castrate resistant prostate cancer patients. Additionally, pre-clinical data in a recent study has found that olaparib may be effective in treating prostate cancer with a *TMPRSS:ERG* mutation [111].

p53 as a Prognostic Marker

The most common genetic cancer mutation, found in more than 50% of human cancers, is a *TP53* gene mutation [112]. The p53 protein is responsible for suspending the cell cycle after DNA damage recognition, activating of DNA repair proteins, and initiating apoptosis. In 25-40% of prostate cancer patients, there is a deletion in the *TP53* locus, and a point mutation in 5-40% of prostate cancer samples [110]. The central dogma for the past few years has been that a *TP53* mutation drove late stage prostate cancer progression [66]. In a recent study using genetically engineered mouse models, it was found that a *TP53* mutation might act as an initiating factor in prostate cancer progression [67]. A further trial of over 7000 prostate cancers has shown that different types of p53 loss have a variable impact on prognosis. Prostate cancers with a truncation or deletion of one allele of *TP53* showed a poorer outcome than patients with normal p53 function and the data also showed that these patients presented with a less aggressive disease than those with homozygous p53 inactivation.

Interestingly though, patients found to have one mutated allele of *TP53* were found to have a far worse prognosis than those with one inactive allele [68]. In a very recent study, an analysis of mutations specifically associated with metastasis in prostate cancer has revealed an enrichment of DNA repair and *TP53* missense mutations, and additional sequencing of metastases from an independent cohort (n=19) has demonstrated that the late acquisition of *TP53* mutations in primary tumour subclones is linked with expansion of subclones with metastatic potential [31]. These findings suggest that mutated forms of p53 are dominant-negative or even oncogenic, as has been shown in breast and lung cancer, and that profiling the variety of p53 mutations found in prostate cancer may have prognostic benefit.

CONCLUSION

Currently, it can be considered that there are two approaches to mutation directed treatment. The first, which can be described as screen based, aims to use genetic sequencing technology to screen the entirety of a person's genome and seeks to identify the individual driving mutations of the cancer to which there may or may not be a therapeutic agent. The second, which can be described as drug-based, designs a screen to search a person's cancer genome for mutations for which there exist therapeutic agents. The drug-based approach enables the restriction of a search to relevant mutations and enables time and resources

to be saved whilst efficiently determining whether or not a particular patient can be aided by treatment with one of the currently available targeted therapeutics, whilst the screen-based approach takes more time and resources whilst holding the possibility that the driving mutation may as yet be untreatable. Limiting the search in favour of drug-based medicine, however, proposes the chance that a new driving mutation could be overlooked. One such screen-based program is already underway. The Michigan Oncology Sequencing Project (MI-ONCOSEQ) aims to bring individualised treatment to patients with advanced cancer by using whole genome sequencing to search a tumour for targetable mutations. The project began in 2011 as a pilot to discover the feasibility of using genomic sequencing in routine clinical care and is still continuing today [113]. Results of this project have so far allowed the MI- ONCOSEQ group to also characterise a number of interesting biological interactions in a number of cancers that offer potentially novel targets for targeted therapeutics [114-116].

Despite the weaknesses of both approaches, they both pose immense benefits in terms of cancer treatment. Therapeutics would be tailored to a patient, and even if resistance were to arise, it is possible that the patient could be re-screened and the appropriate therapeutics could be administered to overcome this. However there are a number of pitfalls to utilizing targeted therapies in the treatment of prostate cancer, the foremost of which is often the design of clinical trials. Many clinical trials that have been mentioned in this review draw conclusions from trials of mutation- specific drugs that are hampered by the absence of testing patients for mutational status [48, 49, 98-100]. Designing a screen based clinical trial would enable a transition into targeted therapies in the treatment of prostate cancer, and could also potentially lead to an indication of the key drivers in prostate cancer. Over the years, this road of mutation targeted therapy has become more accessible due to the decrease in genome sequencing cost and time, and an increase in the data output. Combined with bioinformatics, key mutations in an individual's cancer can be readily identified and known therapeutics can be administered. As global rates of prostate cancer continue to climb and the issue of over-treatment of the disease continues to exist, a tailored approach to an individual's treatment promises to be a boon to both clinicians and prostate cancer patients worldwide.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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REFERENCES

[1] Rahman, N.; Stratton, M.R. The genetics of breast cancer susceptibility. *Annu Rev Genet* **1998**; 32: 95-121.

[2] Edwards, S.M.; Evans, D.G.; Hope, Q.; Norman, A.R.; Barbachano, Y.; Bullock, S.; Kote-Jarai, Z.; Meitz, J.; Falconer, A.; Osin, P.; Fisher, C.; Guy, M.; Jhavar, S.G.; Hall, A.L.; O'Brien, L.T.; Gehr-Swain, B.N.; Wilkinson, R.A.; Forrest, M.S.; Dearnaley, D.P.; Ardern-Jones, A.T.; Page, E.C.; Easton, D.F.; Eeles, R.A.; Collaborators, U.K.G.P.C.S.; Oncology, B.S.o. Prostate cancer in BRCA2 germline mutation carriers is associated with poorer prognosis. *British journal of cancer* **2010**; 103(6): 918-24.

[3] Moody, C.A.; Laimins, L.A. Human papillomavirus oncoproteins: pathways to transformation. *Nature reviews Cancer* **2010**; 10(8): 550-60.

[4] Carey, R.N.; Driscoll, T.R.; Peters, S.; Glass, D.C.; Reid, A.; Benke, G.; Fritschi, L. Estimated prevalence of exposure to occupational carcinogens in Australia (2011-2012). *Occupational and environmental medicine* **2014**; 71(1): 55-62.

[5] Venter, J.C.; Adams, M.D.; Myers, E.W.; Li, P.W.; Mural, R.J.; Sutton, G.G.; Smith, H.O.; Yandell, M.; Evans, C.A.; Holt, R.A.; Gocayne, J.D.; Amanatides, P.; Ballew, R.M.; Huson, D.H.; Wortman, J.R.; Zhang, Q.; Kodira, C.D.; Zheng, X.H.; Chen, L.; Skupski, M.; Subramanian, G.; Thomas, P.D.; Zhang, J.; Gabor Miklos, G.L.; Nelson, C.; Broder, S.; Clark, A.G.; Nadeau, J.; McKusick, V.A.; Zinder, N.; Levine, A.J.; Roberts, R.J.; Simon, M.; Slayman, C.; Hunkapiller, M.; Bolanos, R.; Delcher, A.; Dew, I.; Fasulo, D.; Flanigan, M.; Florea, L.; Halpern, A.; Hannenhalli, S.; Kravitz, S.; Levy, S.; Mobarry, C.; Reinert, K.; Remington, K.; Abu-Threideh, J.; Beasley, E.; Biddick, K.; Bonazzi, V.; Brandon, R.; Cargill, M.; Chandramouliswaran, I.; Charlab, R.; Chaturvedi, K.; Deng, Z.; Di Francesco, V.; Dunn, P.; Eilbeck, K.; Evangelista, C.; Gabrielian, A.E.; Gan, W.; Ge, W.; Gong, F.; Gu, Z.; Guan, P.; Heiman, T.J.; Higgins, M.E.; Ji, R.R.; Ke, Z.; Ketchum, K.A.; Lai, Z.; Lei, Y.; Li, Z.; Li, J.; Liang, Y.; Lin, X.; Lu, F.; Merkulov, G.V.; Milshina, N.; Moore, H.M.; Naik, A.K.; Narayan, V.A.; Neelam, B.; Nusskern, D.; Rusch, D.B.; Salzberg, S.; Shao, W.; Shue, B.; Sun, J.; Wang, Z.; Wang, A.; Wang, X.; Wang, J.; Wei, M.; Wides, R.; Xiao, C.; Yan, C.; Yao, A.; Ye, J.; Zhan, M.; Zhang, W.; Zhang, H.; Zhao, Q.; Zheng, L.; Zhong, F.; Zhong, W.; Zhu, S.; Zhao, S.; Gilbert, D.; Baumhueter, S.; Spier, G.; Carter, C.; Cravchik, A.; Woodage, T.; Ali, F.; An, H.; Awe, A.; Baldwin, D.; Baden, H.; Barnstead, M.; Barrow, I.; Beeson, K.; Busam, D.; Carver, A.; Center, A.; Cheng, M.L.; Curry, L.; Danaher, S.; Davenport, L.; Desilets, R.; Dietz, S.; Dodson, K.; Doup, L.; Ferriera, S.; Garg, N.; Gluecksmann, A.; Hart, B.; Haynes, J.; Haynes, C.; Heiner, C.; Hladun, S.; Hostin, D.; Houck, J.; Howland, T.; Ibegwam, C.; Johnson, J.; Kalush, F.; Kline, L.; Koduru, S.; Love, A.; Mann, F.; May, D.; McCawley, S.; McIntosh, T.; McMullen, I.; Moy, M.; Moy, L.; Murphy, B.; Nelson, K.; Pfannkoch, C.; Pratts, E.; Puri, V.; Qureshi, H.; Reardon, M.; Rodriguez, R.; Rogers, Y.H.; Romblad, D.; Ruhfel, B.; Scott, R.; Sitter, C.; Smallwood, M.; Stewart, E.; Strong, R.; Suh, E.; Thomas, R.; Tint, N.N.; Tse, S.; Vech, C.; Wang, G.; Wetter, J.; Williams, S.; Williams, M.; Windsor, S.; Winn-Deen, E.; Wolfe, K.; Zaveri, J.; Zaveri, K.; Abril, J.F.; Guigo, R.; Campbell, M.J.; Sjolander, K.V.; Karlak, B.; Kejariwal, A.; Mi, H.; Lazareva, B.; Hatton, T.; Narechania, A.; Diemer, K.; Muruganujan, A.; Guo, N.; Sato, S.; Bafna, V.; Istrail, S.; Lippert, R.; Schwartz, R.; Walenz, B.; Yooseph, S.; Allen, D.; Basu, A.; Baxendale, J.; Blick, L.; Caminha, M.; Carnes-Stine, J.; Caulk, P.; Chiang, Y.H.; Coyne, M.; Dahlke, C.; Mays, A.; Dombroski, M.; Donnelly, M.; Ely, D.; Esparham, S.; Fosler, C.; Gire, H.; Glanowski, S.; Glasser, K.; Glodek, A.; Gorokhov, M.; Graham, K.; Gropman, B.; Harris, M.; Heil, J.; Henderson, S.; Hoover, J.; Jennings, D.; Jordan, C.; Jordan, J.; Kasha, J.; Kagan, L.; Kraft, C.; Levitsky, A.; Lewis, M.; Liu, X.; Lopez, J.; Ma, D.; Majoros, W.; McDaniel, J.; Murphy, S.; Newman, M.; Nguyen, T.; Nguyen, N.; Nodell, M.; Pan, S.; Peck, J.; Peterson, M.; Rowe, W.; Sanders, R.; Scott, J.; Simpson, M.; Smith, T.; Sprague, A.; Stockwell, T.; Turner, R.; Venter, E.; Wang, M.; Wen, M.; Wu, D.; Wu, M.; Xia,

Science **2001**; 291(5507): 1304-51.

[6] Lander, E.S.; Linton, L.M.; Birren, B.; Nusbaum, C.; Zody, M.C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; Funke, R.; Gage, D.; Harris, K.; Heaford, A.; Howland, J.; Kann, L.; Lehoczky, J.; LeVine, R.; McEwan, P.; McKernan, K.; Meldrim, J.; Mesirov, J.P.; Miranda, C.; Morris, W.; Naylor, J.; Raymond, C.; Rosetti, M.; Santos, R.; Sheridan, A.; Sougnez, C.; Stange-Thomann, N.; Stojanovic, N.; Subramanian, A.; Wyman, D.; Rogers, J.; Sulston, J.; Ainscough, R.; Beck, S.; Bentley, D.; Burton, J.; Clee, C.; Carter, N.; Coulson, A.; Deadman, R.; Deloukas, P.; Dunham, A.; Dunham, I.; Durbin, R.; French, L.; Grafham, D.; Gregory, S.; Hubbard, T.; Humphray, S.; Hunt, A.; Jones, M.; Lloyd, C.; McMurray, A.; Matthews, L.; Mercer, S.; Milne, S.; Mullikin, J.C.; Mungall, A.; Plumb, R.; Ross, M.; Shownkeen, R.; Sims, S.; Waterston, R.H.; Wilson, R.K.; Hillier, L.W.; McPherson, J.D.; Marra, M.A.; Mardis, E.R.; Fulton, L.A.; Chinwalla, A.T.; Pepin, K.H.; Gish, W.R.; Chissoe, S.L.; Wendl, M.C.; Delehaunty, K.D.; Miner, T.L.; Delehaunty, A.; Kramer, J.B.; Cook, L.L.; Fulton, R.S.; Johnson, D.L.; Minx, P.J.; Clifton, S.W.; Hawkins, T.; Branscomb, E.; Predki, P.; Richardson, P.; Wenning, S.; Slezak, T.; Doggett, N.; Cheng, J.F.; Olsen, A.; Lucas, S.; Elkin, C.; Uberbacher, E.; Frazier, M.; Gibbs, R.A.; Muzny, D.M.; Scherer, S.E.; Bouck, J.B.; Sodergren, E.J.; Worley, K.C.; Rives, C.M.; Gorrell, J.H.; Metzker, M.L.; Naylor, S.L.; Kucherlapati, R.S.; Nelson, D.L.; Weinstock, G.M.; Sakaki, Y.; Fujiyama, A.; Hattori, M.; Yada, T.; Toyoda, A.; Itoh, T.; Kawagoe, C.; Watanabe, H.; Totoki, Y.; Taylor, T.; Weissenbach, J.; Heilig, R.; Saurin, W.; Artiguenave, F.; Brottier, P.; Bruls, T.; Pelletier, E.; Robert, C.; Wincker, P.; Smith, D.R.; Doucette-Stamm, L.; Rubenfield, M.; Weinstock, K.; Lee, H.M.; Dubois, J.; Rosenthal, A.; Platzer, M.; Nyakatura, G.; Taudien, S.; Rump, A.; Yang, H.; Yu, J.; Wang, J.; Huang, G.; Gu, J.; Hood, L.; Rowen, L.; Madan, A.; Qin, S.; Davis, R.W.; Federspiel, N.A.; Abola, A.P.; Proctor, M.J.; Myers, R.M.; Schmutz, J.; Dickson, M.; Grimwood, J.; Cox, D.R.; Olson, M.V.; Kaul, R.; Raymond, C.; Shimizu, N.; Kawasaki, K.; Minoshima, S.; Evans, G.A.; Athanasiou, M.; Schultz, R.; Roe, B.A.; Chen, F.; Pan, H.; Ramser, J.; Lehrach, H.; Reinhardt, R.; McCombie, W.R.; de la Bastide, M.; Dedhia, N.; Blocker, H.; Hornischer, K.; Nordsiek, G.; Agarwala, R.; Aravind, L.; Bailey, J.A.; Bateman, A.; Batzoglou, S.; Birney, E.; Bork, P.; Brown, D.G.; Burge, C.B.; Cerutti, L.; Chen, H.C.; Church, D.; Clamp, M.; Copley, R.R.; Doerks, T.; Eddy, S.R.; Eichler, E.E.; Furey, T.S.; Galagan, J.; Gilbert, J.G.; Harmon, C.; Hayashizaki, Y.; Haussler, D.; Hermjakob, H.; Hokamp, K.; Jang, W.; Johnson, L.S.; Jones, T.A.; Kasif, S.; Kaspryzk, A.; Kennedy, S.; Kent, W.J.; Kitts, P.; Koonin, E.V.; Korf, I.; Kulp, D.; Lancet, D.; Lowe, T.M.; McLysaght, A.; Mikkelsen, T.; Moran, J.V.; Mulder, N.; Pollara, V.J.; Ponting, C.P.; Schuler, G.; Schultz, J.; Slater, G.; Smit, A.F.; Stupka, E.; Szustakowski, J.; Thierry-Mieg, D.; Thierry-Mieg, J.; Wagner, L.; Wallis, J.; Wheeler, R.; Williams, A.; Wolf, Y.I.; Wolfe, K.H.; Yang, S.P.; Yeh, R.F.; Collins, F.; Guyer, M.S.; Peterson, J.; Felsenfeld, A.; Wetterstrand, K.A.; Patrinos, A.; Morgan, M.J.; de Jong, P.; Catanese, J.J.; Osoegawa, K.; Shizuya, H.; Choi, S.; Chen, Y.J.; International Human Genome Sequencing, C. Initial sequencing and analysis of the human genome. *Nature* **2001**; 409(6822): 860-921.

[7] HiSeqX Ten: $1000 Human genome and extreme throughput for population scale sequencing. http://support.illumina.com/sequencing/sequencing_instruments/hiseq-x/documentation.html.

[8] Mardis, E.R. The $1,000 genome, the $100,000 analysis? *Genome medicine* **2010**; 2(11): 84.

[9] Meadows, M. Genomics and personalized medicine. *FDA consumer* **2005**; 39(6): 12-7.

[10] Garrod, A.E. About Alkaptonuria. *Medico-chirurgical transactions* **1902**; 85: 69-78.

[11] Alving, A.S.; Carson, P.E.; Flanagan, C.L.; Ickes, C.E. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* **1956**; 124(3220): 484-5.

[12] Administration, U.F.a.D. *Draft guidance for industry and Food and Drug Administration Staff: In vitro companion diagnostic devices*. [Report], **2011**: 1-13. Available from: http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf). (cited: 25 June **2014**).

[13] Scardino, P.T.; Weaver, R.; Hudson, M.A. Early detection of prostate cancer. *Hum Pathol* **1992**; 23(3): 211-22.

Carroll, P.R. The changing face of low-risk prostate cancer: trends in clinical presentation and primary management. *J Clin Oncol* **2004**; 22(11): 2141-9.

[15] Bastian, P.J.; Gonzalgo, M.L.; Aronson, W.J.; Terris, M.K.; Kane, C.J.; Amling, C.L.; Presti, J.C., Jr.; Mangold, L.A.; Humphreys, E.; Epstein, J.I.; Partin, A.W.; Freedland, S.J. Clinical and pathologic outcome after radical prostatectomy for prostate cancer patients with a preoperative Gleason sum of 8 to 10. *Cancer* **2006**; 107(6): 1265-72.

[16] Grimm, P.; Billiet, I.; Bostwick, D.; Dicker, A.P.; Frank, S.; Immerzeel, J.; Keyes, M.; Kupelian, P.; Lee, W.R.; Machtens, S.; Mayadev, J.; Moran, B.J.; Merrick, G.; Millar, J.; Roach, M.; Stock, R.; Shinohara, K.; Scholz, M.; Weber, E.; Zietman, A.; Zelefsky, M.; Wong, J.; Wentworth, S.; Vera, R.; Langley, S. Comparative analysis of prostate-specific antigen free survival outcomes for patients with low, intermediate and high risk prostate cancer treatment by radical therapy. Results from the Prostate Cancer Results Study Group. *BJU international* **2012**; 109 Suppl 1: 22-9.

[17] Klotz, L. Active surveillance for prostate cancer: overview and update. *Current treatment options in oncology* **2013**; 14(1): 97-108.

[18] Berger, M.F.; Lawrence, M.S.; Demichelis, F.; Drier, Y.; Cibulskis, K.; Sivachenko, A.Y.; Sboner, A.; Esgueva, R.; Pflueger, D.; Sougnez, C.; Onofrio, R.; Carter, S.L.; Park, K.; Habegger, L.; Ambrogio, L.; Fennell, T.; Parkin, M.; Saksena, G.; Voet, D.; Ramos, A.H.; Pugh, T.J.; Wilkinson, J.; Fisher, S.; Winckler, W.; Mahan, S.; Ardlie, K.; Baldwin, J.; Simons, J.W.; Kitabayashi, N.; MacDonald, T.Y.; Kantoff, P.W.; Chin, L.; Gabriel, S.B.; Gerstein, M.B.; Golub, T.R.; Meyerson, M.; Tewari, A.; Lander, E.S.; Getz, G.; Rubin, M.A.; Garraway, L.A. The genomic complexity of primary human prostate cancer. *Nature* **2011**; 470(7333): 214-20.

[19] Mok, T.S.; Wu, Y.L.; Thongprasert, S.; Yang, C.H.; Chu, D.T.; Saijo, N.; Sunpaweravong, P.; Han, B.; Margono, B.; Ichinose, Y.; Nishiwaki, Y.; Ohe, Y.; Yang, J.J.; Chewaskulyong, B.; Jiang, H.; Duffield, E.L.; Watkins, C.L.; Armour, A.A.; Fukuoka, M. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *The New England journal of medicine* **2009**; 361(10): 947-57.

[20] Cooper, C.S.; Foster, C.S. Concepts of epigenetics in prostate cancer development. *British journal of cancer* **2009**; 100(2): 240-5.

[21] Yoshimoto, M.; Ding, K.; Sweet, J.M.; Ludkovski, O.; Trottier, G.; Song, K.S.; Joshua, A.M.; Fleshner, N.E.; Squire, J.A.; Evans, A.J. PTEN losses exhibit heterogeneity in multifocal prostatic adenocarcinoma and are associated with higher Gleason grade. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* **2013**; 26(3): 435-47.

[22] Fialkow, P.J. Clonal origin of human tumors. *Annual review of medicine* **1979**; 30: 135-43.

[23] Shattuck, T.M.; Westra, W.H.; Ladenson, P.W.; Arnold, A. Independent clonal origins of distinct tumor foci in multifocal papillary thyroid carcinoma. *The New England journal of medicine* **2005**; 352(23): 2406-12.

[24] Wang, X.; Wang, M.; MacLennan, G.T.; Abdul-Karim, F.W.; Eble, J.N.; Jones, T.D.; Olobatuyi, F.; Eisenberg, R.; Cummings, O.W.; Zhang, S.; Lopez-Beltran, A.; Montironi, R.; Zheng, S.; Lin, H.; Davidson, D.D.; Cheng, L. Evidence for common clonal origin of multifocal lung cancers. *Journal of the National Cancer Institute* **2009**; 101(8): 560-70.

[25] Villers, A.; McNeal, J.E.; Freiha, F.S.; Stamey, T.A. Multiple cancers in the prostate. Morphologic features of clinically recognized versus incidental tumors. *Cancer* **1992**; 70(9): 2313-8.

[26] Wolters, T.; Montironi, R.; Mazzucchelli, R.; Scarpelli, M.; Roobol, M.J.; van den Bergh, R.C.; van Leeuwen, P.J.; Hoedemaeker, R.F.; van Leenders, G.J.; Schroder, F.H.; van der Kwast, T.H. Comparison of incidentally detected prostate cancer with screen-detected prostate cancer treated by prostatectomy. *The Prostate* **2012**; 72(1): 108-15.

[27] Baca, S.C.; Prandi, D.; Lawrence, M.S.; Mosquera, J.M.; Romanel, A.; Drier, Y.; Park, K.; Kitabayashi, N.; MacDonald, T.Y.; Ghandi, M.; Van Allen, E.; Kryukov, G.V.; Sboner, A.; Theurillat, J.P.; Soong, T.D.; Nickerson, E.; Auclair, D.; Tewari, A.; Beltran, H.; Onofrio, R.C.; Boysen, G.; Guiducci, C.; Barbieri, C.E.; Cibulskis, K.; Sivachenko, A.; Carter, S.L.; Saksena, G.; Voet, D.; Ramos, A.H.; Winckler, W.; Cipicchio, M.; Ardlie, K.; Kantoff, P.W.; Berger, M.F.; Gabriel, S.B.; Golub, T.R.; Meyerson, M.; Lander, E.S.; Elemento, O.; Getz, G.; Demichelis, F.; Rubin, M.A.;

Garraway, L.A. Punctuated evolution of prostate cancer genomes. *Cell* **2013**; 153(3): 666-77.

[28] Ibeawuchi, C.; Schmidt, H.; Voss, R.; Titze, U.; Abbas, M.; Neumann, J.; Eltze, E.; Hoogland, A.M.; Jenster, G.; Brandt, B.; Semjonow, A. Genome-wide investigation of multifocal and unifocal prostate cancer-are they genetically different? *International journal of molecular sciences* **2013**; 14(6): 11816-29.

[29] Lindberg, J.; Klevebring, D.; Liu, W.; Neiman, M.; Xu, J.; Wiklund, P.; Wiklund, F.; Mills, I.G.; Egevad, L.; Gronberg, H. Exome sequencing of prostate cancer supports the hypothesis of independent tumour origins. *European urology* **2013**; 63(2): 347-53.

[30] Boyd, L.K.; Mao, X.; Xue, L.; Lin, D.; Chaplin, T.; Kudahetti, S.C.; Stankiewicz, E.; Yu, Y.; Beltran, L.; Shaw, G.; Hines, J.; Oliver, R.T.; Berney, D.M.; Young, B.D.; Lu, Y.J. High-resolution genome-wide copy-number analysis suggests a monoclonal origin of multifocal prostate cancer. *Genes, chromosomes & cancer* **2012**; 51(6): 579-89.

[31] Hong, M.K.H.; Macintyre, G.; Wedge, D.C.; Van Loo, P.; Patel, K.; Lunke, S.; Alexandrov, L.B.; Sloggett, C.; Cmero, M.; Marass, F.; Tsui, D.; Mangiola, S.; Lonie, A.; Naeem, H.; Sapre, N.; Phal, P.M.; Kurganovs, N.; Chin, X.; Kerger, M.; Warren, A.Y.; Neal, D.; Gnanapragasam, V.; Rosenfeld, N.; Pedersen, J.S.; Ryan, A.; Haviv, I.; Costello, A.J.; Corcoran, N.M.; Hovens, C.M. Tracking the origins and drivers of subclonal metastatic expansion in prostate cancer. *Nat Commun* **2015**; 6.

[32] Liu, W.; Laitinen, S.; Khan, S.; Vihinen, M.; Kowalski, J.; Yu, G.; Chen, L.; Ewing, C.M.; Eisenberger, M.A.; Carducci, M.A.; Nelson, W.G.; Yegnasubramanian, S.; Luo, J.; Wang, Y.; Xu, J.; Isaacs, W.B.; Visakorpi, T.; Bova, G.S. Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. *Nature medicine* **2009**; 15(5): 559-65.

[33] Normanno, N.; De Luca, A.; Bianco, C.; Strizzi, L.; Mancino, M.; Maiello, M.R.; Carotenuto, A.; De Feo, G.; Caponigro, F.; Salomon, D.S. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* **2006**; 366(1): 2-16.

[34] Antonicelli, A.; Cafarotti, S.; Indini, A.; Galli, A.; Russo, A.; Cesario, A.; Lococo, F.M.; Russo, P.; Mainini, A.F.; Bonifati, L.G.; Nosotti, M.; Santambrogio, L.; Margaritora, S.; Granone, P.M.; Dutly, A.E. EGFR-targeted therapy for non-small cell lung cancer: focus on EGFR oncogenic mutation. *Int J Med Sci* **2013**; 10(3): 320-30.

[35] Peraldo-Neia, C.; Migliardi, G.; Mello-Grand, M.; Montemurro, F.; Segir, R.; Pignochino, Y.; Cavalloni, G.; Torchio, B.; Mosso, L.; Chiorino, G.; Aglietta, M. Epidermal Growth Factor Receptor (EGFR) mutation analysis, gene expression profiling and EGFR protein expression in primary prostate cancer. *BMC Cancer* **2011**; 11: 31.

[36] Fu, M.; Zhang, W.; Shan, L.; Song, J.; Shang, D.; Ying, J.; Zhao, J. Mutation status of somatic EGFR and KRAS genes in Chinese patients with prostate cancer (PCa). *Virchows Arch* **2014**; 464(5): 575-81.

[37] Marks, R.A.; Zhang, S.; Montironi, R.; McCarthy, R.P.; MacLennan, G.T.; Lopez-Beltran, A.; Jiang, Z.; Zhou, H.; Zheng, S.; Davidson, D.D.; Baldridge, L.A.; Cheng, L. Epidermal growth factor receptor (EGFR) expression in prostatic adenocarcinoma after hormonal therapy: a fluorescence in situ hybridization and immunohistochemical analysis. *The Prostate* **2008**; 68(9): 919-23.

[38] Canil, C.M.; Moore, M.J.; Winquist, E.; Baetz, T.; Pollak, M.; Chi, K.N.; Berry, S.; Ernst, D.S.; Douglas, L.; Brundage, M.; Fisher, B.; McKenna, A.; Seymour, L. Randomized phase II study of two doses of gefitinib in hormone-refractory prostate cancer: a trial of the National Cancer Institute of Canada-Clinical Trials Group. *J Clin Oncol* **2005**; 23(3): 455-60.

[39] Carrion-Salip, D.; Panosa, C.; Menendez, J.A.; Puig, T.; Oliveras, G.; Pandiella, A.; De Llorens, R.; Massaguer, A. Androgen-independent prostate cancer cells circumvent EGFR inhibition by overexpression of alternative HER receptors and ligands. *Int J Oncol* **2012**; 41(3): 1128-38.

[40] Gravina, G.L.; Marampon, F.; Piccolella, M.; Biordi, L.; Ficorella, C.; Motta, M.; Jannini, E.A.; Tombolini, V.; Festuccia, C. Antitumor effects of carnertinib in castration resistant prostate cancer models: A Comparative study with erlotinib. *The Prostate* **2011**.

[41] Chen, L.; Mooso, B.A.; Jathal, M.K.; Madhav, A.; Johnson, S.D.; van Spyk, E.; Mikhailova, M.; Zierenberg-Ripoll, A.; Xue, L.; Vinall, R.L.; deVere White, R.W.; Ghosh, P.M. Dual EGFR/HER2 inhibition sensitizes prostate cancer cells to androgen withdrawal by suppressing ErbB3. *Clin Cancer Res* **2011**; 17(19): 6218-28.

[42] Goldenberg, M.M. Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer. *Clin Ther* **1999**; 21(2): 309-18.

[43] Tagliabue, E.; Campiglio, M.; Pupa, S.M.; Menard, S.; Balsari, A. Activity and resistance of trastuzumab according to different clinical settings. *Cancer Treat Rev* **2012**; 38(3): 212-7.

[44] Siampanopoulou, M.; Galaktidou, G.; Dimasis, N.; Gotzamani-Psarrakou, A. Profiling serum HER-2/NEU in prostate cancer. *Hippokratia* **2013**; 17(2): 108-12.

[45] Guyader, C.; Ceraline, J.; Gravier, E.; Morin, A.; Michel, S.; Erdmann, E.; de Pinieux, G.; Cabon, F.; Bergerat, J.P.; Poupon, M.F.; Oudard, S. Risk of hormone escape in a human prostate cancer model depends on therapy modalities and can be reduced by tyrosine kinase inhibitors. *PLoS One* **2012**; 7(8): e42252.

[46] Carlsson, J.; Shen, L.; Xiang, J.; Xu, J.; Wei, Q. Tendencies for higher co-expression of EGFR and HER2 and downregulation of HER3 in prostate cancer lymph node metastases compared with corresponding primary tumors. *Oncol Lett* **2013**; 5(1): 208-14.

[47] Whang, Y.E.; Armstrong, A.J.; Rathmell, W.K.; Godley, P.A.; Kim, W.Y.; Pruthi, R.S.; Wallen, E.M.; Crane, J.M.; Moore, D.T.; Grigson, G.; Morris, K.; Watkins, C.P.; George, D.J. A phase II study of lapatinib, a dual EGFR and HER-2 tyrosine kinase inhibitor, in patients with castration-resistant prostate cancer. *Urol Oncol* **2013**; 31(1): 82-6.

[48] de Bono, J.S.; Bellmunt, J.; Attard, G.; Droz, J.P.; Miller, K.; Flechon, A.; Sternberg, C.; Parker, C.; Zugmaier, G.; Hersberger-Gimenez, V.; Cockey, L.; Mason, M.; Graham, J. Open-label phase II study evaluating the efficacy and safety of two doses of pertuzumab in castrate chemotherapy-naive patients with hormone-refractory prostate cancer. *J Clin Oncol* **2007**; 25(3): 257-62.

[49] Agus, D.B.; Sweeney, C.J.; Morris, M.J.; Mendelson, D.S.; McNeel, D.G.; Ahmann, F.R.; Wang, J.; Derynck, M.K.; Ng, K.; Lyons, B.; Allison, D.E.; Kattan, M.W.; Scher, H.I. Efficacy and safety of single-agent pertuzumab (rhuMAb 2C4), a human epidermal growth factor receptor dimerization inhibitor, in castration-resistant prostate cancer after progression from taxane-based therapy. *J Clin Oncol* **2007**; 25(6): 675-81.

[50] Douillard, J.Y.; Sobrero, A.; Carnaghi, C.; Comella, P.; Diaz- Rubio, E.; Santoro, A.; Van Cutsem, E. Metastatic colorectal cancer: integrating irinotecan into combination and sequential chemotherapy. *Ann Oncol* **2003**; 14 Suppl 2: ii7-12.

[51] Meyerhardt, J.A.; Mayer, R.J. Systemic therapy for colorectal cancer. *The New England journal of medicine* **2005**; 352(5): 476-87.

[52] Baselga, J. The EGFR as a target for anticancer therapy--focus on cetuximab. *Eur J Cancer* **2001**; 37 Suppl 4: S16-22.

[53] Lièvre, A.; Bachet, J.B.; Le Corre, D.; Boige, V.; Landi, B.; Emile, J.F.; Côté, J.F.; Tomasic, G.; Penna, C.; Ducreux, M.; Rougier, P.; Penault-Llorca, F.; Laurent-Puig, P. KRAS Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer. *Cancer Research* **2006**; 66(8): 3992-95.

[54] Bouali, S.; Chrétien, A.S.; Ramacci, C.; Rouyer, M.; Becuwe, P.; Merlin, J.L. PTEN expression controls cellular response to cetuximab by mediating PI3K/AKT and RAS/RAF/MAPK downstream signalling in KRAS wild-type, hormone refractory prostate cancer cells. *Oncology Reports* **2009**; 21: 731-35.

[55] Festuccia, C.; Angelucci, A.; Gravina, G.L.; Biordi, L.; Millimaggi, D.; Muzi, P.; Vicentini, C.; Bologna, M. Epidermal growth factor modulates prostate cancer cell invasiveness regulating urokinase-type plasminogen activator activity. EGF-receptor inhibition may prevent tumor cell dissemination. *Thrombosis and haemostasis* **2005**; 93(5): 964-75.

[56] Cathomas, R.; Rothermundt, C.; Klingbiel, D.; Bubendorf, L.; Jaggi, R.; Betticher, D.C.; Brauchli, P.; Cotting, D.; Droege, C.; Winterhalder, R.; Siciliano, D.; Berthold, D.R.; Pless, M.; Schiess, R.; von Moos, R.; Gillessen, S.; Swiss Group for Clinical Cancer Research, S. Efficacy of cetuximab in metastatic castration-resistant prostate cancer might depend on EGFR and PTEN expression: results from a phase II trial (SAKK 08/07). *Clin Cancer Res* **2012**; 18(21): 6049-57.

[57] Liu, P.; Cheng, H.; Roberts, T.M.; Zhao, J.J. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nature reviews Drug discovery* **2009**; 8(8): 627-44.

[58] Brugge, J.; Hung, M.C.; Mills, G.B. A new mutational AKTivation in the PI3K pathway. *Cancer cell* **2007**; 12(2): 104-7.

[59] Dowling, R.J.; Topisirovic, I.; Fonseca, B.D.; Sonenberg, N. Dissecting the role of mTOR: lessons from mTOR inhibitors. *Biochimica et biophysica acta* **2010**; 1804(3): 433-9.

[60] Sato, T.; Nakashima, A.; Guo, L.; Coffman, K.; Tamanoi, F. Single amino-acid changes that confer constitutive activation of mTOR are discovered in human cancer. *Oncogene* **2010**; 29(18): 2746-52.

[61] Knight, Z.A.; Shokat, K.M. Chemically targeting the PI3K family. *Biochemical Society transactions* **2007**; 35(Pt 2): 245-9.

[62] Andre, F.; O'Regan, R.; Ozguroglu, M.; Toi, M.; Xu, B.; Jerusalem, G.; Masuda, N.; Wilks, S.; Arena, F.; Isaacs, C.; Yap, Y.S.; Papai, Z.; Lang, I.; Armstrong, A.; Lerzo, G.; White, M.; Shen, K.; Litton, J.; Chen, D.; Zhang, Y.; Ali, S.; Taran, T.; Gianni, L. Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. *The lancet oncology* **2014**; 15(6): 580-91.

[63] Rizzo, M.; Facchini, G.; Savastano, C.; Lorenzo, G.D.; Lucia, L.D.; Maiorino, L.; Casale, B.; Grimaldi, G.; Formato, R.; Febbraro, A.; Carteni, G. Everolimus as second-line therapy for metastatic renal cell carcinoma: a 'real-life' study. *Future oncology* **2014**: 1-6.

[64] cBioPortal for Cancer Genomics. The Cancer Genome Atlas. http://www.cbioportal.org/public-portal/ (cited: 07 August **2014**).

[65] Kosaka, T.; Miyajima, A.; Shirotake, S.; Suzuki, E.; Kikuchi, E.; Oya, M. Long-term androgen ablation and docetaxel up-regulate phosphorylated Akt in castration resistant prostate cancer. *The Journal of urology* **2011**; 185(6): 2376-81.

[66] Bitting, R.L.; Armstrong, A.J. Targeting the PI3K/Akt/mTOR pathway in castration-resistant prostate cancer. *Endocrine-related cancer* **2013**; 20(3): R83-99.

[67] Amato, R.J.; Jac, J.; Mohammad, T.; Saxena, S. Pilot study of rapamycin in patients with hormone-refractory prostate cancer. *Clinical genitourinary cancer* **2008**; 6(2): 97-102.

[68] Armstrong, A.J.; Netto, G.J.; Rudek, M.A.; Halabi, S.; Wood, D.P.; Creel, P.A.; Mundy, K.; Davis, S.L.; Wang, T.; Albadine, R.; Schultz, L.; Partin, A.W.; Jimeno, A.; Fedor, H.; Febbo, P.G.; George, D.J.; Gurganus, R.; De Marzo, A.M.; Carducci, M.A. A pharmacodynamic study of rapamycin in men with intermediate- to high-risk localized prostate cancer. *Clin Cancer Res* **2010**; 16(11): 3057-66.

[69] Sarbassov, D.D.; Ali, S.M.; Sengupta, S.; Sheen, J.H.; Hsu, P.P.; Bagley, A.F.; Markhard, A.L.; Sabatini, D.M. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Molecular cell* **2006**; 22(2): 159-68.

[70] Yasumizu, Y.; Miyajima, A.; Kosaka, T.; Miyazaki, Y.; Kikuchi, E.; Oya, M. Dual PI3K/mTOR inhibitor NVP-BEZ235 sensitizes docetaxel in castration resistant prostate cancer. *The Journal of urology* **2014**; 191(1): 227-34.

[71] Park, H.S.; Hong, S.K.; Oh, M.M.; Yoon, C.Y.; Jeong, S.J.; Byun, S.S.; Cheon, J.; Lee, S.E.; Moon du, G. Synergistic antitumor effect of NVP-BEZ235 and sunitinib on docetaxel-resistant human castration-resistant prostate cancer cells. *Anticancer research* **2014**; 34(7): 3457-68.

[72] Zhu, W.; Fu, W.; Hu, L. NVP-BEZ235, dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor, prominently enhances radiosensitivity of prostate cancer cell line PC-3. *Cancer biotherapy & radiopharmaceuticals* **2013**; 28(9): 665-73.

[73] Hong, S.W.; Shin, J.S.; Moon, J.H.; Kim, Y.S.; Lee, J.; Choi, E.K.; Ha, S.H.; Lee, D.H.; Chung, H.N.; Kim, J.E.; Kim, K.P.; Hong, Y.S.; Lee, J.L.; Lee, W.J.; Choi, E.K.; Lee, J.S.; Jin, D.H.; Kim, T.W. NVP-BEZ235, a dual PI3K/mTOR inhibitor, induces cell death through alternate routes in prostate cancer cells depending on the PTEN genotype. *Apoptosis : an international journal on programmed cell death* **2014**; 19(5): 895-904.

[74] Ali, I.U.; Schriml, L.M.; Dean, M. Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. *Journal of the National Cancer Institute* **1999**; 91(22): 1922-32.

[75] Chen, M.; Pratt, C.P.; Zeeman, M.E.; Schultz, N.; Taylor, B.S.; O'Neill, A.; Castillo-Martin, M.; Nowak, D.G.; Naguib, A.; Grace, D.M.; Murn, J.; Navin, N.; Atwal, G.S.; Sander, C.; Gerald, W.L.; Cordon-Cardo, C.; Newton, A.C.; Carver, B.S.; Trotman, L.C. Identification of PHLPP1 as a tumor suppressor reveals the role of feedback activation in PTEN-mutant prostate cancer progression. *Cancer cell* **2011**; 20(2): 173-86.

[76] McMenamin, M.E.; Soung, P.; Perera, S.; Kaplan, I.; Loda, M.; Sellers, W.R. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. *Cancer Res* **1999**; 59(17): 4291-6.

[77] Giri, D.; Ittmann, M. Inactivation of the PTEN tumor suppressor gene is associated with increased angiogenesis in clinically localized prostate carcinoma. *Hum Pathol* **1999**; 30(4): 419-24.

[78] Lotan, T.L.; Gurel, B.; Sutcliffe, S.; Esopi, D.; Liu, W.; Xu, J.; Hicks, J.L.; Park, B.H.; Humphreys, E.; Partin, A.W.; Han, M.; Netto, G.J.; Isaacs, W.B.; De Marzo, A.M. PTEN protein loss by immunostaining: analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. *Clin Cancer Res* **2011**; 17(20): 6563-73.

[79] Halvorsen, O.J.; Haukaas, S.A.; Akslen, L.A. Combined loss of PTEN and p27 expression is associated with tumor cell proliferation by Ki-67 and increased risk of recurrent disease in localized prostate cancer. *Clin Cancer Res* **2003**; 9(4): 1474-9.

[80] Chen, Z.; Trotman, L.C.; Shaffer, D.; Lin, H.K.; Dotan, Z.A.; Niki, M.; Koutcher, J.A.; Scher, H.I.; Ludwig, T.; Gerald, W.; Cordon-Cardo, C.; Pandolfi, P.P. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature* **2005**; 436(7051): 725-30.

[81] Naguib, A.; Trotman, L.C. PTEN plasticity: how the taming of a lethal gene can go too far. *Trends in cell biology* **2013**; 23(8): 374-9.

[82] Shaw, A.T.; Yeap, B.Y.; Mino-Kenudson, M.; Digumarthy, S.R.; Costa, D.B.; Heist, R.S.; Solomon, B.; Stubbs, H.; Admane, S.; McDermott, U.; Settleman, J.; Kobayashi, S.; Mark, E.J.; Rodig, S.J.; Chirieac, L.R.; Kwak, E.L.; Lynch, T.J.; Iafrate, A.J. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* **2009**; 27(26): 4247-53.

[83] Shaw, A.T.; Kim, D.W.; Nakagawa, K.; Seto, T.; Crino, L.; Ahn, M.J.; De Pas, T.; Besse, B.; Solomon, B.J.; Blackhall, F.; Wu, Y.L.; Thomas, M.; O'Byrne, K.J.; Moro-Sibilot, D.; Camidge, D.R.; Mok, T.; Hirsh, V.; Riely, G.J.; Iyer, S.; Tassell, V.; Polli, A.; Wilner, K.D.; Janne, P.A. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *The New England journal of medicine* **2013**; 368(25): 2385-94.

[84] Choi, Y.L.; Soda, M.; Yamashita, Y.; Ueno, T.; Takashima, J.; Nakajima, T.; Yatabe, Y.; Takeuchi, K.; Hamada, T.; Haruta, H.; Ishikawa, Y.; Kimura, H.; Mitsudomi, T.; Tanio, Y.; Mano, H.; Group, A.L.K.L.C.S. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *The New England journal of medicine* **2010**; 363(18): 1734-9.

[85] Shen, L.; Ji, H.F. More on crizotinib. *The New England journal of medicine* **2011**; 364(8): 777-8.

[86] Sun, H.Y.; Ji, F.Q. A molecular dynamics investigation on the crizotinib resistance mechanism of C1156Y mutation in ALK. *Biochemical and biophysical research communications* **2012**; 423(2): 319-24.

[87] Drake, J.M.; Graham, N.A.; Lee, J.K.; Stoyanova, T.; Faltermeier, C.M.; Sud, S.; Titz, B.; Huang, J.; Pienta, K.J.; Graeber, T.G.; Witte, O.N. Metastatic castration-resistant prostate cancer reveals intrapatient similarity and interpatient heterogeneity of therapeutic kinase targets. *Proc Natl Acad Sci U S A* **2013**; 110(49): E4762-9.

[88] de Klein, A.; van Kessel, A.G.; Grosveld, G.; Bartram, C.R.; Hagemeijer, A.; Bootsma, D.; Spurr, N.K.; Heisterkamp, N.; Groffen, J.; Stephenson, J.R. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. *Nature* **1982**; 300(5894): 765-7.

[89] Heisterkamp, N.; Stam, K.; Groffen, J.; de Klein, A.; Grosveld, G. Structural organization of the bcr gene and its role in the Ph' translocation. *Nature* **1985**; 315(6022): 758-61.

[90] McWhirter, J.R.; Galasso, D.L.; Wang, J.Y. A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. *Mol Cell Biol* **1993**; 13(12): 7587-95.

[91] Nowell, P.C.; Hungerford, D.A. Chromosome studies on normal and leukemic human leukocytes. *Journal of the National Cancer Institute* **1960**; 25: 85-109.

[92] Buchdunger, E.; Zimmermann, J.; Mett, H.; Meyer, T.; Muller, M.; Druker, B.J.; Lydon, N.B. Inhibition of the Abl protein-tyrosine kinase *in vitro* and *in vivo* by a 2-phenylaminopyrimidine derivative. *Cancer Res* **1996**; 56(1): 100-4.

[93] Kelley, R.K.; Hwang, J.; Magbanua, M.J.; Watt, L.; Beumer, J.H.; Christner, S.M.; Baruchel, S.; Wu, B.; Fong, L.; Yeh, B.M.; Moore, A.P.; Ko, A.H.; Korn, W.M.; Rajpal, S.; Park, J.W.; Tempero, M.A.; Venook, A.P.; Bergsland, E.K. A phase 1 trial of imatinib, bevacizumab, and metronomic cyclophosphamide in advanced colorectal cancer. *British journal of cancer* **2013**; 109(7): 1725-34.

[94] Gambacorti-Passerini, C.; Antolini, L.; Mahon, F.X.; Guilhot, F.; Deininger, M.; Fava, C.; Nagler, A.; Della Casa, C.M.; Morra, E.; Abruzzese, E.; D'Emilio, A.; Stagno, F.; le Coutre, P.; Hurtado-Monroy, R.; Santini, V.; Martino, B.; Pane, F.; Piccin, A.; Giraldo, P.; Assouline, S.; Durosinmi, M.A.; Leeksma, O.; Pogliani, E.M.;