Abstract

Breast cancer is projected to be the most common cancer in women in 2020 in the United States. Despite high remission rates, treatment side-effects remain an issue, hence the interest in novel approaches such as immunotherapies which aim to utilise patients' immune systems to target cancer cells. This review summarises the basics of breast cancer including staging and treatment options, followed by a discussion on immunotherapy, including immune checkpoint blockade. After this, examples of the role of omics-type data and computational biology/bioinformatics in breast cancer are explored. Ultimately, there are several promising areas to investigate such as the prediction of neoantigens and the use of multi-omics data to direct research, with noted appropriate in clinical trial design~~,~~ in terms of endpoints.

Key Words

Breast Cancer Immunotherapy Omics Multi-omics data Computational Biology Bioinformatics Neoantigen

Main Body

Introduction to Breast Cancer

In the past quarter of a century the prevalence of breast cancer has increased by approximately 40%, and it is projected to be the most common cancer in women in 2020 in the United States [1-3]. Breast cancer can be classified based on ~~,~~the grade of the cancer cells, the stage of the tumour, and specific gene/protein markers & molecular pathology, with particular emphasis on the latter in recent years [4, 5]~~, with~~. ~~most~~ Most breast cancers or associated benign neoplasms ~~beginning~~ begin in the ducts or lobules of the breast [6], with n~~.~~ ~~N~~on-invasive breast neoplasms ~~can be~~ classified as lobular carcinoma in situ (LCIS) or ductal carcinoma in situ (DCIS) [7]. LCIS is seen as a risk factor for breast cancer development, whilst the malignant transformation for DCIS may take longer. Development of LCIS or DCIS into invasive cancers (invasive lobular carcinoma and invasive/infiltrating carcinoma respectively) is characterised by overall lack of architecture and haphazard tissue organisation [7].

Risk factors for breast cancer include age, personal and family history of breast cancer, reproductive milestones that increase a woman's oestrogen exposure, exogenous hormone use, and genetic factors such as BRCA1/BRCA2 mutations [7]. Breast cancer may often present asymptomatically or may be detected by patients as they notice palpable breast lumps. The often-asymptomatic nature of the disease necessitates early detection, as survival outcomes are very different depending on the cancer's stage. More than 90% of breast cancers at the time of diagnosis are not metastatic [8], which tallies with the fact that 90% of breast cancer diagnoses have an overall survival rate of five years or more, despite the fact that only 15% of patients diagnosed with Stage IV are alive five years later. Thus, early detection and appropriate staging is crucial.

This review outlines the molecular classification of breast cancer, along with how it is staged at the clinical level. After discussing existing treatment options, the review then proceeds to discuss novel emerging approaches for breast cancer, focussing on immunotherapy, and the role that multi-omics data can have in this. The review then closes with a "Future Perspectives" section, which details where it is anticipated this field could evolve in the coming years.

Molecular Classification of Breast Cancer

Breast cancer, overall, is commonly divided into four main molecular subtypes: luminal A, luminal B, triple negative, and human epidermal growth factor receptor 2 (HER2)-enriched. Luminal A is characterised by being oestrogen receptor (ER) and/or progesterone receptor (PR) positive, HER2 negative, and possessing low expression levels of Ki67, a key protein driving breast cancer cell proliferation [9]. Comparatively, luminal B is characterised by possessing higher levels of Ki67 and may be HER2 positive or negative, whilst HER2-enriched breast cancers are ER and PR negative but HER2 positive [10]. Lastly, triple-negative breast cancer (TNBC) accounts for approximately 15% of all breast cancers [11] and is characterised by a lack of ER and PR expression alongside a lack of (HER2 amplification [11]. The lack of these key targets naturally leads to the poor prognosis of these patients, as targeted and endocrine therapies are not currently available for TNBC patients [11]. Understanding the molecular subtype of breast cancer is crucial as it has been shown to play a role in response to immunotherapies, for example different molecular subtypes may exhibit immune evasion through different mechanisms [12], which may lead to stratified treatment regimens in the future.

Breast Cancer Staging

Breast cancer staging will primarily depend on the primary tumour site and size (T), the extent of regional lymph node involvement (N), and the existence of distant metastasis (M) [13-16]. The latest 8th edition of the American Joint Committee on Cancer (AJCC) staging system also incorporates the use of other biomarkers such as oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) for prognostic staging [17], which is also recommended in the National Institute for Health and Care Excellence (NICE) guidelines. NICE states that ER, PR and HER2 statuses of invasive breast cancers should be requested at the point of initial diagnosis confirmed by histological findings [18]. AJCC 8th edition also endorsed the use of multigene assays such as Oncotype Dx® and Mammaprint® in predicting prognosis for hormone receptor positive, HER2-negative breast cancer, while there is a lack of evidence to support the use of such tool in triple negative cancers and HER2-positive cancers [14]. Further work is, however, required to illustrate the additional value provided by Oncotype Dx as a recent analysis questioned the applicability of such multigene assay in a broader population [19].

Current Standard Therapy for Breast Cancer

Neoadjuvant therapies such as chemotherapy, radiotherapy and hormonal therapy aim to reduce tumour size and avoid mastectomies, and are provided prior to breast conserving surgeries (BCS) [20]. Neoadjuvant chemotherapy has been reported to be more effective than neoadjuvant hormonal therapy in ER+, HER2- cancers [21], though it was also acknowledged and highlighted the need to develop predictive biomarkers to guide neoadjuvant therapies [21]. Furthermore, it has also been advised that caution should be used in clinical practice surrounding the use of neoadjuvant therapies, as a recent meta-analysis reported that the use of neoadjuvant chemotherapy may have elevated risk of relapse than those who received adjuvant chemotherapy [22]. This was further supported by a March 2020 study which found that specific risk factors such as young age, TNBC, and node-positive tumours were associated with an elevated risk of locoregional recurrence after neoadjuvant chemotherapy [23]. There are further issues surrounding the use of neoadjuvant therapies, including (until recently) limited evidence in the literature comparing efficacies of different neoadjuvant therapies and a lack of standardisation for pathologic evaluation of post-neoadjuvant breast cancer specimens in the routine clinical setting [24].

Following initial treatment with neoadjuvant chemotherapy, NICE recommends either mastectomy or BCS followed by radiotherapy for those suffering from locally advanced or inflammatory breast cancer [18]. It has been shown that patients who received BCS with radiotherapy had significantly higher overall survival and disease-specific survival than patients undergoing mastectomy, and that this was particularly true for patients aged over 50 with hormone receptor-positive tumours against patients under 50 with hormone-receptor negative tumours [25].

The NICE guidelines also state that further surgeries should be offered after BCS in cases of invasive breast cancer or DCIS located at the radial margins. The primary goal in the surgical management of invasive breast cancer, in the absence of detectable lymph node involvement on ultrasound, is to accurately stage the axilla using sentinel lymph node biopsy (SLNB). Alternatively, axillary lymph node dissection (ALND) should be offered where there is evidence of metastatic lymphadenopathy on histopathology or sentinel lymph node macro-metastases following SLNB [18]. Radiotherapy should be offered to most patients with invasive breast cancer following BCS unless they have been assessed to have significantly lower absolute risk of relapse and will comply to a minimum 5 year course of adjuvant hormonal therapy [18]. Per NICE guidelines, chemotherapy is part of the adjuvant treatment of invasive breast cancer when justified by significant risk. This normally consists of a treatment regimen involving a taxane and an anthracycline [18]. In addition, depending on hormonal receptor and HER2 statuses, chemotherapy is also used in advanced breast cancer as a monotherapy or combined with immunotherapies such as monoclonal antibodies [26]. Figure 1 below summarises the management algorithm for early and locally advanced breast cancer:

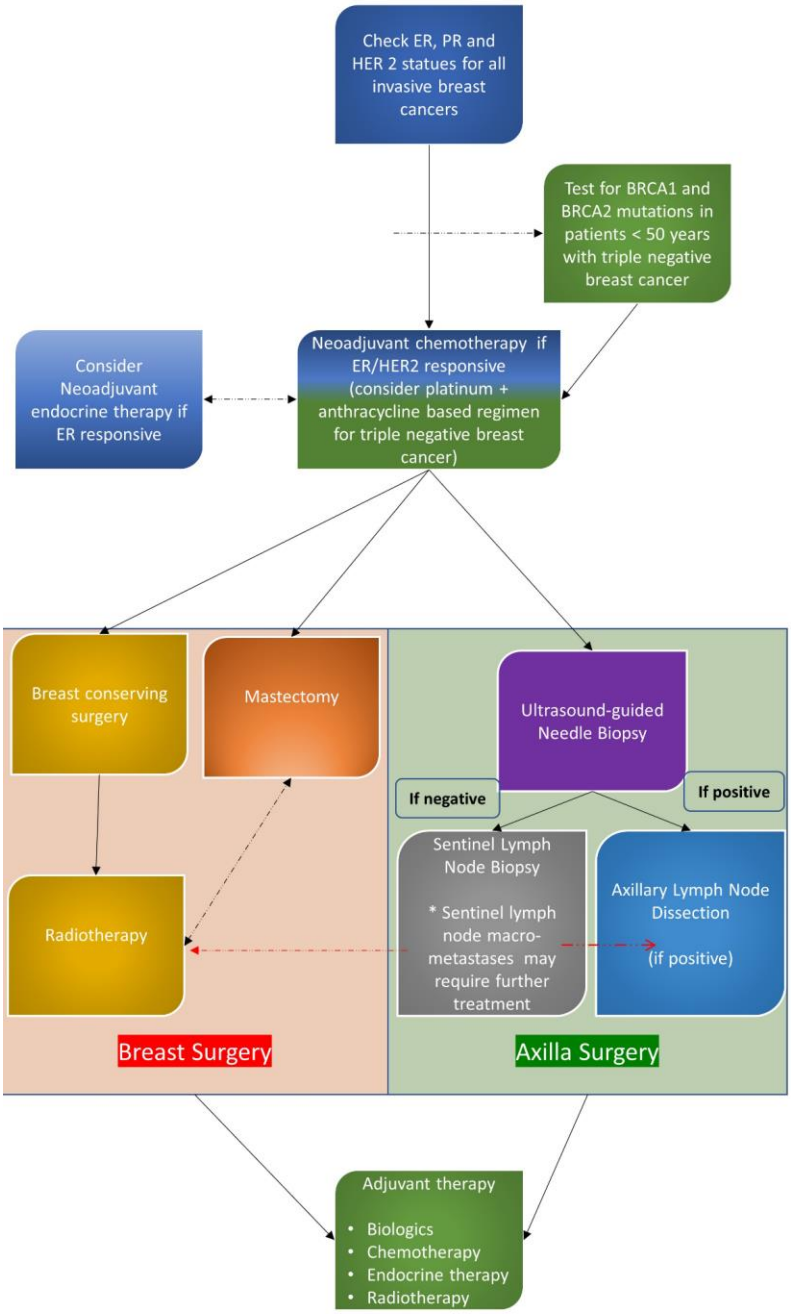
Check ER, PR and HER 2 statues for all invasive breast cancers
Test for BRCA1 and BRCA2 mutations in patients < 50 years with triple negative breast cancer
Consider Neoadjuvant endocrine therapy if ER responsive
Neoadjuvant chemotherapy if ER/HER2 responsive (consider platinum + anthracycline based regimen for triple negative breast cancer)
Breast conserving surgery
Mastectomy
Radiotherapy
Ultrasound-guided Needle Biopsy
If negative
If positive
Sentinel Lymph Node Biopsy
* Sentinel lymph node macro-metastases may require further treatment
Axillary Lymph Node Dissection
(if positive)
Breast Surgery
Axilla Surgery
Adjuvant therapy
Biologics
Chemotherapy
Endocrine therapy
Radiotherapy

Figure 1: Overview of the management algorithm for early and locally advanced breast cancer. Information adapted from the NICE guidelines for early and locally advanced breast cancer [18].

Despite the above and overall good success rates for breast cancer remission, the treatments are of course associated with unpleasant side effects and not all patients are successfully treated. Thus, there has been an interest in developing alternative treatments that are more targeted, less toxic, and less invasive.

Photothermal Therapy

Phototherapy (also known as light therapy) is the exposure of various forms of light for treating a number of conditions, which has been applied both clinically and experimentally in oncology. A key advantage of phototherapy, in general, is that it is non-invasive and can have high spatiotemporal precision [27]. The two main kinds of phototherapy to consider are photodynamic therapy (PDT), which involves a particular type of light along with a light-sensitive drug treatment (photosensitiser) or nanoparticle [28], and photothermal therapy (PTT) which, unlike photodynamic therapy, does not depend on oxygen for functionality [27]. PTT agents instead absorb light energy and convert it to heat to generate their anti-cancer effects [27, 29].

Liang and colleagues demonstrated the potential efficacy of PTT via an erythrocyte membrane-coated black phosphorus quantum dot formulation (BPQD-RMN) which reduced basal-like breast tumour growth in-vivo [30]. Whilst there are a number of examples of the application of phototherapy to breast cancer research, of particular interest here is the potential to combine phototherapy with immunotherapy. As will be expanded upon further on in the review, programmed cell death 1 (PD-1) is a key immune checkpoint marker that is a frequent target for immunotherapy. The study by Liang and colleagues demonstrated that residual and metastatic tumour growth was delayed *in vivo* when BPQD-RMN-mediated phototherapy was combined with an antibody against PD-1, as a result of reduced $CD8^+$ T cell exhaustion [30, 31]. Thus, combining phototherapy with other promising approaches such as immunotherapy could offer further therapeutic benefits.

Introduction to Immunotherapy

Immunotherapy is a constantly evolving field aiming to encourage the immune system to target tumour cells more effectively while intending to bypass the side effects of existing treatment options [32]. There are various treatment opportunities branching out from immunotherapy. Some examples of these include immunotherapeutic vaccinations, immune checkpoint blockade, oncolytic virotherapy and antitumor monoclonal antibodies [33]. Cancer immunotherapy can be categorised into active and passive immunity. Immunotherapeutic injection is one of the most common examples of active immunotherapy. This results in stimulation of a prolonged immune response and memory. In contrast is passive immunotherapy which produces short-lived responses requiring regular administration of the treatment, due to not activating the immune system in a systematic manner in situ and relying on isolated effectors activated in vitro [34, 35].

One of the challenges associated with immunotherapy is resistance to treatment, more specifically acquired resistance, in which immunotherapy initially demonstrated success initially with the cancer, but it relapsed after some time had passed [36]. Immunogenic in nature, T-lymphocytes constitute the majority (70-80%) of immune cells in breast cancer tissue. B-lymphocytes, macrophages, antigen presenting cells and natural killer (NK) cells account for the remaining immune cells in breast cancer tissue [37]. Tumours expressing human epidermal growth factor receptor 2 (HER2), along with triple-negative breast cancer (TNBC), can be viewed as the most immunogenic subtypes of breast cancer. Various immunotherapy strategies are currently being investigated for treatment of breast cancer [38].

Immunotherapeutic vaccinations for breast cancer

Vaccines can be separated into two categories: prophylactic (preferred for cancers with an infectious aetiology) or therapeutic (aim to treat an existing disease by utilising a patient's immune system) [33, 39]. In general, vaccines developed for cancer aim to initiate type I CD 4+ or 8+ T cell responses [33, 39]. In general, vaccines developed for cancer aim to initiate type I CD 4+ or 8+ T cell responses [33, 39]. In general, vaccines developed for cancer aim to initiate type I CD 4+ or 8+ T cell responses against tumour associated antigens (TAAs) [40]. Breast cancer has associations with various TAAs such as HER2 and Mucin 1 (MUC1). Approximately 20% of breast cancer patients express HER2 and overexpression of mucin especially MUC1, MUC3 and MUC4 is commonly observed in breast cancer. Targeting the previously

mentioned sites have shown clinical benefit [37, 41, 42]. Figure 2 below summarizes the various type of vaccination tested for breast cancer:

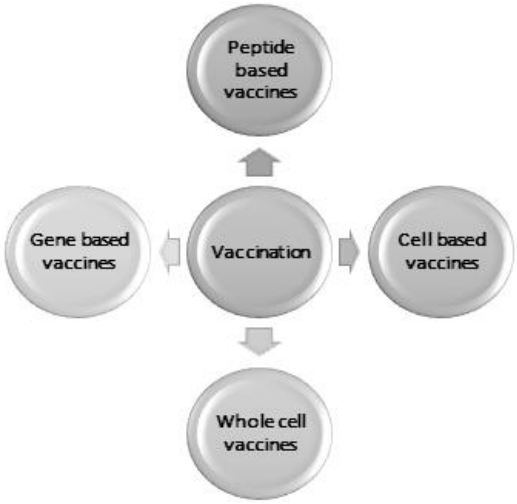

Figure 2: Immunotherapeutic vaccination in breast cancer.

Peptide-based vaccines

As clarified earlier, HER2 (ERBB2) expression is associated with an increased mortality and

HER2 derivatives such as E75 HER2 (a derivative of the extracellular domain) may also be useful for

MUC1

Belonging to the mucoprotein family, MUC1 is a high molecular weight glycoprotein which is increasingly expressed among various carcinomas such as ovarian and breast cancer. Expression of certain tumour associated carbohydrate residues including sialyl-Tn (STn) antigens (+ Thomsen-Freidenreich) are a consequence of aberrant glycosylation [37, 40]. Poor prognosis of breast cancer can be linked to high levels of STn [51]. A Phase III trial involved the administration of STn vaccine conjugated to keyhole-limpet Hemocyanin (KLH) (also referred to as Theratope). This study recruited 1028 women with MBC. Despite it being well tolerated, Theratope administration had no effect on overall survival (OS) or time to progression duration. However, simultaneous administration of

Theratope and endocrine therapy resulted in a statistically significant OS difference [51]. Another study administered a poxviral vaccines, PANVAC, which contained transgenes for MUC-1, CEA and 3 T-cell co-stimulatory molecules. There were no grade 3 or 4 toxicities observed with PANVAC administration. This study benefited patients with limited tumour burden or those with minimal history of chemotherapy [37, 52]. NCT03524261 is an upcoming phase II clinical trial involving the use of activated CIK and CD3-MUC1 biospecific antibodies in the treatment of breast cancer.

Cell-based vaccines

Autologous cell-based

Lapuleucel-T (APC8024) is formulated from a combination of peripheral mononuclear cells incorporating antigen-presenting cells with BA7072 (recombinant fusion protein) [37, 53]. A phase I study was conducted to assess the benefit of lapuleucel-T in 19 patients with MBC overexpressing HER2. This therapy was well-tolerated with pyrexia (74%) and rigors (58%) as the most common adverse effects. Lymphocyte proliferation and interferon gamma enzyme-linked immunospot assay measured a noteworthy immune response following administration with above treatment. Furthermore, 3 patients reported stable disease lasting ≥ 1 year [53].

Dendritic cell (DC)-based

A study administered DCs pulsed with either HER2/MUC-1 in patients with ovarian (3/10) and breast cancer (7/10). DC vaccine was well tolerated, and 5/10 patients peripheral blood sample contained cytotoxic T-lymphocytes specific to a peptide. This study obtained results that showed potential for DCs pulsed with a single antigen in treatment of ovarian and breast cancer [54]. One clinical trial involved the administration of dendritic cells vaccinations pulsed with HER2 were stimulated in vitro with interferon gamma (IFN and bacterial lipopolysaccharide [55]. This process allowed that the dendritic cells to exude increased levels of interleukin-12p70. Increased levels of sensitisation towards peptides including IFN γ secreting CD4 and 8 were observed. 7/11 patient's surgical tumour samples revealed lower levels of HER2 expression [55]. This trial displayed potential in treating early stage breast cancer. However further trials are required to draw conclusions about the safety and efficacy of DC based vaccinations for breast cancer. NCT04105582 is an on-going phase I trial aiming to vaccinate TNBC patients with autologous DCs. NCT03384914 is another on-going clinical trial

intending to evaluate the safety and efficacy of two vaccines (DC1 and WOKVAC) in both, female and male patients with breast cancer.

In summary, vaccines developed for prevention or treatment of breast cancer have not been approved yet. Modifications of various factors such as route of delivery, timing of vaccine administration, patient selection and optimal combination of therapies could enhance the effectiveness of breast cancer vaccines [56]. Additionally, the use of high-throughput methodologies, as discussed further on in the manuscript, can aid in the identification of neoantigens that may facilitate vaccine development, with the added benefit of neoantigens being tumour-specific antigens (TSAs) rather than tumour associated. This is of key importance, as the specific nature of the TSA results in a negligible risk of triggering severe adverse-related events due to non-specificity, in contrast to TAAs [57].

Immune checkpoint blockade

Located on T-cell surfaces, immune checkpoint receptors communicate either positive or negative signals to T cells. Cytotoxic T-lymphocyte antigen-4 (CTLA-4), Programmed cell death 1 (PD-1) and lymphocyte activation gene 3 (LAG-3) are some examples inhibiting T cell immune responses. Conversely, OX40 is a receptor that stimulates T-cell activity via positive signals [38]. The development of inhibitory antibodies aimed at immune checkpoints has shown promise in the treatment of various cancers such as breast cancer, bladder cancer and melanoma [37].

CTLA-4 Inhibitors

Tremelimumab and ipilimumab are examples of anti-CTLA-4 monoclonal antibodies that are being tested for breast cancer treatment [24]. Apart from stimulating T-cell activation, tremelimumab aims to prevent CTLA4 binding to CD80 and CD86. A phase I study recruited 26 patients with ER-positive advanced breast cancer and administered tremelimumab plus exemestane to establish their efficacy in breast cancer treatment and judge tumour or immune responses. This combination was tolerated in patients with the majority experiencing grade I or II toxicities. Diarrhoea (46%) and pruritus (42%) were two of the most common adverse effects. This trial revealed stable disease in 11 patients (42%) for ≥12 weeks. Additionally, increased levels of inducible costimulator plus T cells were observed which most likely resulted in immune activation secondary to CTLA-4 blockade [58]. In a pilot study, ipilimumab with or without cryoablation was administered to 19 patients with early stage breast

cancer. Cryoablation with ipilimumab revealed a limited increase in the ratio of CD8+Ki67 tumour T cells to T-regulatory cells. Treatment with ipilimumab alone resulted in activated T-cells in the bloodstream [59]. Table 1 summarises ongoing clinical trials for breast cancer treatment involving anti-CTLA-4 monoclonal antibodies:

Table 1: Ongoing clinical trials with anti-CTLA-4 monoclonal antibodies in breast cancer treatment

Clinical Trial Identifier	Phase	Study title	Estimated participants (n)	Intervention	Status	Responsible party
NCT04185311	I	Ipilimumab, Nivolumab, and Talimogene Laherparepvec before surgery in treating participants with localized, triple-negative or estrogen receptor positive, HER2 negative breast cancer-deleted	20	Ipilimumab, Nivolumab, Talimogene Laherparepvec	Recruiting	Jonsson Comprehensive Cancer Center
NCT03608865	II	Durvalumab (MEDI4736) and Tremelimumab in hormone receptor-positive, hypermutated MBC identified by whole exome sequencing	30	Durvalumab, Tremelimumab	Recruiting	Yonsei University
NCT02536794	II	MEDI4736 and Tremelimumab in treating patients with metastatic HER2 negative breast cancer	30	Anti-B7H1 monoclonal antibody MEDI4736, Tremelimumab	Recruiting	Northwestern University

NCT03430466	II	Anti PD-L1 antibody + Anti-CTLA-4 antibody in combination with hormone therapy in patients with hormone receptor positive, HER2 negative, recurrent or MBC	33	Durvalumab, Tremelimumab, Fulvestrant	Recruiting	Kyoto Breast Cancer Research Network
NCT02639026	I	Trial of hypofractionated radiotherapy in combination with MEDI4736 and Tremelimumab for patients with metastaic melanoma and lung, breast and pancreatic cancers	30	Radiotherapy, MEDI4736, Tremelimumab	Recruiting	Abramson Cancer Center of the University of Pennsylvania
NCT03518606	I/II	Metronomic oral Vinorelbine plus ANTI-PD-L1/Anti-CTLA-4 immunotherapy in patients with advanced solid tumours (MOVIE)	150	Durvalumab, Tremelimumab, metronomic Vinorelbine	Recruiting	UNICANCER
NCT02643303	I/II	A phase 1/ 2 study of in situ vaccination with Tremelimumab and IV Durvalumab plus PolyICLC in subjects with advanced, measurable biopsy-	102	Durvalumab, Tremelimumab, Poly ICLC	Recruiting	Ludwig Institute for Cancer Research

		accessible cancers				
NCT03674827	I	A study to evaluate escalating doses of a vaccine-based immunotherapy regimen for NSCLC and TNBC	97	PF-06936308	Recruiting	Pfizer
NCT03789110	II	NIMBUS: Nivolumab plus Ipilimumab in metastatic hypermutated HER2-ngeative breast cancer	30	Nivolumab, Ipilimumab	Recruiting	Sara Tolaney, Dana-Farber Cancer Institute
NCT02453620	I	Entinostat, Nivolumab and Ipilimumab in treating patients with solid tumours that are metastatic or cannot be removed by surgery or locally advanced or metastatic HER2-negative breast cancer	45	Entinostat, Ipilimumab, Nivolumab	Recruiting	National Cancer Institute
NCT02563925	Pilot study	Brain irradiation and Tremelimumab in MBC	28	Tremelimumab, Durvalumab, Brain radiotherapy or stereotactic radiosurgery	Active, not recruiting	Memorial Sloan Kettering Cancer Center
NCT03132467	(Early) I	Durvalumab and Tremelimumab before surgery in treating patients with hormone receptor	15	Durvalumab, Tremelimumab	Active, not recruiting	M.D. Anderson Cancer Center

		positive, HER2 negative Stage II-III breast cancer				
NCT02527434	II	Study of tremelimumab in patients with advanced solid tumours	64	Tremelimumab, MEDI4736	Active, not recruiting	AstraZeneca
NCT02997995	II	Durvalumab and endocrine therapy in ER+/HER2- breast cancer after CD8+ infiltration effective immune-attractant exposure (ULTIMATE)	240	Tremelimumab, Durvalumab, Biopsy	Active, not recruiting	UNICANCER
NCT03982173	II	Basket trial for combination therapy with Durvalumab (Anti-PDL1) (MEDI4736) and Tremelimumab (Anti-CTLA4) in patients with metastatic solid tumours (MATILDA)	88	Tremelimumab, Durvalumab	Active, not recruiting	Gustave Roussy, Cancer Campus, Grand Paris
NCT01975831	I	A phase 1 study to evaluate MEDI4736 in combination with Tremelimumab	106	MEDI4736, Tremelimumab	Active, not recruiting	Ludwig Institute for Cancer Research

LAG-3

A phase I/II trial demonstrated the application of chemoimmunotherapy in 30 patients with MBC. A soluble kind of LAG-3, IMP321 (LAG-31g) was administered in combination with paclitaxel to these patients [38, 60]. IMP321 helps to prolong an initial immune response that is initiated by chemotherapy application. The combined regimen was well tolerated, and clinical benefit was seen at 6 months for 90% of patients. An increase in the both, quantity and activation of antigen

presenting cells plus an increase in the amount of NK and CD8 T cells was attributed to the application of IMP321 [60]. Further phase II/III trials are necessary to confirm the advantage of chemoimmunotherapy application in breast cancer patients. NCT02614833 is an active phase II clinical trial assessing IMP321 with standard chemotherapy paclitaxel in patients with hormone receptor-positive breast cancer.

PD-1 & PD-L1 Inhibitors

Expressed on activated NK cells, monocytes, dendritic cells, T cells, B cells and myeloid cells, PD-1 is an inhibitory immune checkpoint receptor. PD-L1 (CD274 or B7-H1) and PD-L2 (CD273 or B7-DC) are the main ligand partners for PD-1. Negative regulation of T-cells is a consequence of PD-1/PD-L1 expression. Approximately 20-30% of breast cancer patients, especially those with TNBC, exhibit PD-L1 [37, 38, 61]. A phase Ib nonrandomised trial was conducted to assess the application of pembrolizumab (MK-3475) in patients with TNBC. Arthralgia (18.8%), fatigue (18.8%), myalgia (18.8%) and nausea (15.6%) were the most common adverse effects observed. 15.6% of patients enrolled experienced one or more grade 3-5 adverse event. Pembrolizumab administration exhibited an overall response rate (ORR) of 18.5% comprising of one complete response. However, the treatment(s) patients received prior to the trial and high-LDL levels of patient must be considered when reviewing the ORR [61]. NCT02447003 is an on-going phase II trial to assess pembrolizumab monotherapy in patients with metastatic TNBC. NCT02054806 is another on-going phase I clinical trial assessing the application of pembrolizumab among patients with incurable advanced biomarker positive solid tumours. A humanized monoclonal antibody, Atezolizumab (MPDL3280A) functions to rebuild tumour associated T-cell immunity through inhibition of PD-L1 to PD-1. Atezolizumab plus nab-paclitaxel was administered to 11 patients with metastatic TNBC. This combination was well-tolerated, and its promising results encouraged an on-going phase III trial of atezolizumab with nab-paclitaxel among previously untreated patients with metastatic TNBC (ClinicalTrials.gov identifier: NCT02425891) [62]. NCT01633970 is another on-going clinical trial involving atezolizumab administration with bevacizumab and/or chemotherapy among patients with locally advanced or metastatic solid tumours. Durvalumab (anti-PD-L1) and nivolumab (anti-PD-1) are other inhibitors being tested for breast cancer treatment.

Although immunotherapy holds much promise, clinical trial results should always be interpreted with a healthy dose of caution. Many clinical trials will utilise progression-free survival (PFS) as an endpoint on the trial, sometimes alongside but sometimes without overall survival (OS). In any case,

results reported with surrogate endpoints such as PFS should be interpreted cautiously, as research has shown that immunotherapeutic treatment effect sizes with PFS were 17% greater, on average, than with OS [63]. In the case of breast cancer, only little to moderate correlation (surrogacy) between PFS and OS has been shown, which is also true for other surrogate endpoints such as disease control and time to progression [64, 65]. In the case of immune-checkpoint blockade, the majority of the focus is on CTLA-4 and PD-1/PDL-1, though it has rightly been argued that there are other immune checkpoints that can be focussed upon , and indeed high-throughput 'omics' methodologies can help with this [66].

'Omics' Data and Computational Biology

Improved technologies and research methodologies have allowed for the examination of biological data in an integrated, cohesive, and holistic manner, by looking at a much wider facet of the biology than traditional laboratory experimentation would have allowed. In particular, the use of 'omics' technologies (genomics, proteomics, metabolomics, etc) allows for exceptionally large amounts of data to be generated and analysed. Through the use of computational biology it is possible to integrate this data and analyse it, and also utilise it for other purposes. Systems biology is a field that aims to accurately model biological data, particularly for entire systems, and this can be done for key cancer proteins such as TP53 (mutated in over half of human cancers) [67]. This kind of modelling may also be performed for nuclear hormone receptors such as the oestrogen receptor, which is evidently important for breast cancer therapy. The proof of principle for this came from the 2017 paper which modelled the glucocorticoid hormone receptor for leukaemia [68].

Both this TP53 model and the GR model were Boolean *in silico* simulations of the protein interaction networks surrounding each protein. These models were capable of generating predictions (following *in silico* mutations) as to how the network would operate following an *in vivo* loss-of-function mutation, and model validation by literature mining and experimental data demonstrated good prediction rates for each, above that of a random model. In particular, these models were capable of being integrated with high-throughput experimental data, and were each validated by microarray data from both cancer cell lines in the laboratory harbouring the mutations simulated or from cancer patients [67, 68]. In the case of the GR model, preliminary model validation demonstrated potential correlation of the model's prediction of the disease's severity with the survival length of patients, though the authors noted it should be interpreted cautiously and preliminarily [68].

Analysis of these types of models were taken further forward by integrating mesothelioma patient RNA-seq data with the TP53 model [69]. Data from the study by Bueno and colleagues [70] which looked at transcriptomes and exomes, was utilised for this. The RNA-seq data from 71 mesothelioma patients was collectively analysed, and patients were subgrouped based on factors such as treatment and mutation status. By combining this data with the model analysis, the authors were able to identify particular up- and down-regulated genes that represented therapeutic targets in different subpopulations. These targets were then verified through *in vitro* laboratory methodologies. Thus, the development of these models represents a way to utilise the high-throughput data that exists and may be a step towards personalised therapy.

It is certainly possible that this kind of model could be built for genes/proteins key to breast cancer , and superimposition of cell-line or patient-based omics data to such a model would no doubt provide similar therapeutic insights to breast cancer signalling, much as the GR model did for leukaemia and the re-analysis of the TP53 model did for mesothelioma [67-69]. Should such models be built for breast cancer it would no doubt provide novel insights into breast cancer signalling following the use of *in silico* mutations and the use of omics-type data to validate predictions.

Application of Multi-Omics Data to Breast Cancer

Further to the above-described application of omics-type data to computational models, it is also Further to the above-described application of omics-type data to computational models, it is also Further to the above-described application of omics-type data to computational models, it is also Further to the above-described application of omics-type data to computational models, it is also important to consider the benefits of looking at different types of omics data collectively rather than individually, such as analysing transcriptome, epigenome and proteome data concurrently to generate an overall conclusion. The use of multi-omics data in oncology from a general perspective has been comprehensively reviewed by Chakraborty and colleagues [71]. In principle, whilst there is increasing complexity from genome through to proteome and single-type omics data can provide highly useful information (for instance mutation identification), the complexity of cancer at many levels requires a deeper level of investigation than single-omics data can provide [71]. A simple example would be that mutations identified in whole-genome DNA sequencing may not have any importance due to lack of

expression, which is something a proteomics screen could identify – or alternatively an epigenome screen could identify if a wild-type gene is instead inactive through epigenetic mechanisms. Thus, integration of multiple types of omics data allows for a wider holistic view of cancer. An example of the importance of multi-omics data is through a

In the case of breast cancer, whilst there have been several multi-omics studies, there are notably fewer that are specifically related to immunotherapy. Thus, a number of multi-omics approaches are discussed herein in the wider context, rather than directing focus specifically to its application to immunotherapy, though there are studies that relate to specific elements such as the tumour microenvironment and neoantigen prediction, the latter of which is of course important for therapeutic vaccine development.

A second 2016 study generated proteomic and phosphoproteomic data to identify the functional consequence of somatic mutations identified at the genome level, highlighting potential therapeutic targets and narrowing candidate nominations for driver genes [73]. The study by Mertins and colleagues also highlighted the importance of a multi-omics approach through their identification of a G-protein-coupled receptor cluster in the phosphoproteome data that was not easily identifiable at the mRNA level [73]. In this study, genomic and transcriptomic variants were identified at the peptide level through searching high-resolution accurate-mass tandem mass spectrometry (MS/MS) data that were not matched to RefSeq against a patient-specific sequence database. Notably, although RNA-seq detected them only as single transcript reads, this MS/MS approach identified a number of splice isoforms, along with several single amino acid variants, splice junctions, and frameshifts [73]. Ultimately, however, the number of genomic and transcriptomic variants confirmed by MS/MS as peptides was low. The study by Mertins and colleagues provided further evidence of the benefit of integrated analysis, as their study identified that protein kinases such as CDK12, often encompassed in the ERBB2 (HER2) amplicon, were upregulated at the RNA, protein, and phosphoprotein level and showed similar gene-amplification-driven proteogenomic patterns to HER2 [73]. Given the continuing search for druggable targets such as tyrosine kinases, the identification of CDK12 as highly active in the majority of HER2-positive tumours is of interest [73]

The importance of metabolism in cancer, most famously highlighted via the Warburg effect (usage of aerobic glycolysis), lends itself to multi-omics investigation. Terunuma and colleagues undertook a metabolomics approach and identified 2-hydroxyglutarate (2-HG) accumulation in a subset of

tumour types which was also linked to aberrant MYC activation [74]. The study also found that 2-HG could promote cellular proliferation and inhibit apoptosis. Auslander and colleagues conducted an integrated analysis of metabolome and transcriptome data to develop a model that predicted metabolite levels based on the expression level of the enzymes catalysing them [75]. This prediction method was then analysed against a large dataset of breast cancer patients to estimate the importance of the metabolite concentration for patient survival. The depletion of key cancer-related metabolites such as glucose and acetate appeared to be significantly correlated with improved patient survival [75]. The authors also argued that the limited possibilities of metabolomic targeting leads to the possibility of their prediction pipeline having utility in deciphering the role of different metabolites in cancer progression and prediction of biomarkers for early detection and prognosis. This thus demonstrates additional usage of multi-omics approaches.

There have been several studies published in 2020 that relate to multi-omics data. Huang and colleagues, in May 2020, released an article detailing an integrated genome-wide DNA methylation analysis, protein profile analysis, and metabolme analysis on MCF-7 breast cancer cells treated with estradiol (E2, an oestrogen) and/or sulforaphane (SFN) (a chemopreventive phytochemical) [76]. Interestingly, this study found that combined E2 and SFN treatment showed a similar DNA methylation profile to the control cells, indicating that the chemoprotective effects of SFN may arise through reversing the effects of E2 [76]. Among other results, when the DNA methylation, protein and metabolite analyses were integrated by annotating the methylated genes and proteins with Gene Ontology (GO) terms and metabolic pathway enrichment identified by Reactome. By using KEGG to map metabolites to the corresponding pathways, the authors were able to locate the common pathway of genes, proteins and metabolites [76]. This process also identified differentially methylated genes and differentially expressed proteins, and the approach identified that the reversal effects of SFN were associated with glutathione metabolism and purine metabolism. Ultimately the integrated approach provided a 'blueprint' of the relationship of the biological molecules at different stages, facilitating the mechanistic understanding of chemopreventive medicines [76], and is a further example of the use of multiple data in concurrence demonstrating deeper insight.

A fundamental driver anti-cancer therapy is of course an understanding of apoptotic pathways and the key players therein. Again in May 2020, Wang and colleagues sought to identify the spatial pattern of BCL2 gene family members within the context of chromatin using correlations between gene expression, gene alteration, and chromatin accessibility, all related to clinical outcomes in

gynaecologic and breast cancer [77]. The study focussed on integrating multi-omics data available from a number of sources cBioPortal, Gene Expression Profiling Interactive Analysis (GEPIA), and assay of transposase-accessible chromatin with sequencing datasets available from the UCSC Xena browser [77]. Focussing on gynaecological tumours, the authors found that differential BCL2 family member expression was paired with widespread chromatin accessibility changes, in addition to the authors noting a relationship between 'gene expression, gene amplification, enhancer signatures, DNA methylation and overall patient survival' [77]. The study identified clinical correlations with genes such as BAD, BIK and BAK1 via prognostic analysis. Whilst this study focussed on gynaecologic cancers, such an approach could certainly be adopted for different types of breast cancer to generate equivalent results.

Karim and colleagues, in January 2020, undertook a multi-omics pan-cancer approach to analyse the importance of bone morphogenic protein 5 (BMP5), due to conflicting literature [78]. To do this, a number of resources were utilised including (but not limited to) ONCOMINE (mRNA analysis) [79], Gene Expression across Normal and Tumor tissue (GENT, http://gent2.appex.kr/gent2/) [80], UALCAN (used for the expression pattern of BMP5 mRNA, but can also analyse promoter methylation, correlation, and prognosis) [81], PrognoScan (determination of potential tumor biomarkers and therapeutic targets) [82] and cBioPortal [83, 84], which contains a range of data including mRNA level and DNA methylation data. The integration of the numerous sets of data allowed the authors to come to a more robust conclusion, identifying via a prognostic analysis that there was a negative association of BMP5 downregulation with four types of cancer, including breast [78]. Whilst it is possible to use multi-omics approaches to look at the 'big picture' or at molecular signatures, for example, such data may also be useful to conduct a sort of meta-analysis of a number of different datasets to come to key conclusions surrounding one gene in different contexts.

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Cui and colleagues recently undertook a multi-omics approach investigating expression data of long non-coding RNAs (lncRNAs), microRNAs, mRNA, as well as methylation levels and somatic mutations, along with patient survival data following drug treatment [85]. The aim was to evaluate the drug responses of patients from a multi-omics perspective, to gain a wider understanding. The study identified drug response-related lncRNAs (DRIncs) through their integrated analysis, which is of potential therapeutic benefit through increased understanding of the molecular mechanisms underlying drug response [85]. This study represents a strong example of applying multi-omics data to understand molecular response at numerous levels.

In relation to the immune system, multi-omics data has also been utilised to investigate the tumour microenvironment (TME), which consists not only of cancer cells but also other cell types such as tumour-infiltrating immune cells, secreted factors such as cytokines and non-cellular elements of the extracellular matrix (ECM) [86]. The TME is known to be important for breast cancer development and progression, and also offers the opportunity for therapeutic targeting [87]. From an immunotherapy perspective, the fact that most patients do not respond to immune checkpoint blockade, despite its potential and inclusion on patient regimens, highlights the need to look at the TME in further detail for its involvement in this [86]. Multi-omics profiling of the TME has been reviewed in more detail by Finotello and Eduati [86].

Xiao and colleagues, on behalf of the AME Breast Cancer Collaborative Group, recently conducted a large study involving extensive immunogenomic analysis to identify the heterogeneity and prognostic significance of the tumour microenvironment in triple-negative breast cancer [88], on the back of data indicating lack of success for immunotherapies for TNBC despite its higher immunogenicity than other breast cancers [88]. 386 TNBC samples were analysed involving a range of data sources including OncoScan microarray copy-number data (n = 335), tumour RNA-seq data (n = 245; paired normal tissues: n = 90), hematoxylin and eosin (H & E) sections data (n = 300), whole-exome sequencing data (n = 268), tissue microarray data (n = 181) and HTA 2.0 microarray data (n = 141) [88].

From the above, the TNBC microenvironments were classified into three heterogenous clusters: “cluster 1, the “immune-desert” cluster, with low microenvironment cell infiltration; cluster 2, the “innate immune-inactivated” cluster, with resting innate immune cells and nonimmune stromal cells infiltration; and cluster 3, the “immune-inflamed” cluster, with abundant adaptive and innate immune cells infiltration” [88]. It was found by Xiao and colleagues that the clusters had significant prognostic potential, with cluster 1 being unable to attract immune cells, cluster 2 demonstrating features that possibly contribute to immune evasion, whilst cluster 3 featured high expression of immune checkpoint molecules [88]. Such analysis may pave the way forward for patient stratification for immunotherapy trials, by specifically selecting patients exhibiting a similar profile to “cluster 3”.

Similar to the above is the study by McGrail and colleagues [89], which aimed to investigate the causes behind immune invasion in recurrent copy number alteration-driven cancers such as breast cancer, as the levels of cytotoxic T-lymphocytes (CTLs) does not correlate with neoantigen

expression in these cancer types. McGrail and colleagues utilised an integrated multi-omics framework inclusive of proteomic data, gene expression data, genetic mutation phosphor-/total-proteomics along with interactome networks. This study unveiled that CTL levels in breast cancer correlate with ATM expression, and that ATM expression was linked to elevated cytokine secretion, in turn promoting CTL infiltration [89]. The identification of driving factors of immune infiltration is key to improving existing immunotherapeutics, and thus this study highlights another example of how multi-omics data may be beneficial for pushing breast cancer therapies forward.

Multi-omics profiling has also been employed to investigate differences in molecular signatures between different patient cohorts [90]. Kan and colleagues compared whole exomes and transcriptomes of a Korean breast cancer patient cohort (primarily pre-menopausal) to a primarily Caucasian and post-menopausal breast cancer patient cohort available from the TCGA [90]. Curiously, it was found that the Korean cohort had a larger proportion of HER2-enriched and luminal B molecular subtypes, and a lower proportion of luminal A with lower ER expression [90]. The same patient cohort also demonstrated a higher level of mutation in *BRCA1*, *BRCA2*, and *TP53*, and were also found, overall, to have higher levels of tumour-infiltrating lymphocytes (TILs) and higher expression of PD-L1 [90]. This study therefore clearly demonstrates the importance of identifying molecular signatures associated with particular molecular subtypes of cancer and with particular patient cohorts, as knowledge of this may further promote stratified patient treatment. It also is concurrent with the knowledge that TIL density correlates with PD-L1 expression in breast cancer [91].

A key aspect of the response to immunotherapy is reportedly the tumour mutational burden (TMB). The role of TMB in relation to breast cancer has been comprehensively analysed in March 2020 by Barroso-Sousa and colleagues using sequencing data of 3969 patients [92]. In principle, high tumour burden was found in only 5% of breast cancer cases and was more common in metastatic tumours; however, it was proposed that those patients that exhibit hypermutation were more likely to benefit from immune checkpoint blockade in the form of PD-1 inhibition [92]. Hormone receptor-negative and HER2-negative tumours were found to have a higher level of tumour mutational burden than HER2-enriched tumours, which in turn had higher mutational burden than hormone receptor-positive and HER2-negative tumours [92].

In the same study, APOBEC activity and mismatch repair deficiency (MMRd) were the most common mutational processes in the hypermutated tumours at 59.2% and 36.4% respectively[92]. APOBEC

represents a crucial area of study, as APOBEC has been shown to generate mutational signatures in breast cancer, and is associated with higher tumour mutational burden [93]. Additionally, APOBEC has been fundamentally identified as a key enzymatic driver of mutation in breast cancer, promoting C-T mutations [94]. Chen and colleagues conducted an integrated genomic and association analysis using data from ten cancer types from TCGA, investigating *APOBEC3A* and *APOBEC3B*, APOBEC-mutational signature, germline *APOBEC3A/B* deletions, neoantigen loads, and TILs [93]. A key finding from the study was that germline *APOBEC3A/B* deletion resulted in elevated APOBEC mutational signature, higher proportion of $CD8^+$ T cells in TILs, and elevated neoantigen load in breast cancer [93].

Neoantigen Prediction

Neoantigen expression is considered an important determinant in the response to immunotherapy, and the use of multi-omics data to consider the wider TME and its relationship to tumour cells can help to uncover relationships between tumour cells and immune cells. For instance, RNA-seq data has been utilised in-conjunction with whole-genome or exome data to predict patient-specific neoantigens [86]. In this context, the use of the word neoantigen means a peptide resulting from a somatic mutation present in the cancer cells and hence not expressed on healthy cells, leading to targeted therapy. As is clear from the above section, high tumour mutational burden is associated with elevated levels of neoantigens [92] It follows logically that high levels of neoantigens correlate with improved response to immunotherapies, and this does indeed seem to be the case for immune checkpoint blockade [95-97], though in the case of breast cancer immune infiltration does not correlate with neoantigen levels [89]. Matching tumour omics data with equivalent data from normal cells can allow for the identification of neo-antigens on a patient-by-patient basis [95]. These identified (or predicted) neoantigens then need to be ranked according to their likelihood to induce a T-cell response.

Despite the potential of this approach, as of August 2019 there was no formalised consensus approach and few best practice guidelines [95] for this procedure. However, generally speaking, after the analysis of matched DNA sequence data to identify somatic mutations, human leukocyte antigen (HLA) haplotyping should be performed to determine the patient's HLA alleles and their corresponding MHC class. RNA-seq data can then be used to calculate the expression levels of neoantigens before ranking. There are many bioinformatics tools utilised to perform these procedures, reviewed by Richters and colleagues [95]. A key benefit of neoantigen prediction is the

possibility of deriving novel immunotherapy vaccinations, the most frequent of which are synthetic long peptide (SLP), tumour cell, nucleic acid, and dendritic cell (DC)-based vaccines [98] The promise of this approach of neoantigen identification/prediction is evident due to there being several clinical trials currently investigating neoantigens, with examples summarised below in Table 2:

Table 2: Clinical trials involving neoantigens in breast cancer that are either ongoing, recruiting, or not yet recruiting. All data taken from clinicaltrials.gov. Note that some trials are investigating cancers other than breast concurrently.

Clinical Trial Identifier	**Phase**	**Study title**	**Estimated participants (n)**	**Intervention**	**Status**	**Responsible party**
NCT04105582	I	Phase I Clinical Study of Immunotherapy With Personalized Synthetic Vaccines in Patients With Triple Negative Breast Cancer	5	Neo-antigen pulsed Dendritic cell	Recruiting	Universidad Nacional de Colombia
NCT03199040	I	A Randomized Phase 1 Trial of Neoantigen DNA Vaccine Alone vs. Neoantigen DNA Vaccine Plus Durvalumab in Triple Negative Breast Cancer Patients Following Standard of Care Therapy	24	Neoantigen DNA vaccine, Durvalumab	Recruiting	Washington University School of Medicine

NCT03606967	II	Randomized Phase 2 Clinical Trial of Nab-Paclitaxel + MEDI4736 (Durvalumab) + Neoantigen Vaccine vs. Nab-Paclitaxel + MEDI4736 (Durvalumab) in Patients With Metastatic Triple Negative Breast Cancer	70	Drug: Carboplatin Biological: Durvalumab Drug: Gemcitabine Hydrochloride Drug: Nab-paclitaxel Biological: Personalized Synthetic Long Peptide Vaccine Drug: Poly ICLC	Not yet recruiting	National Cancer Institute (NCI)
NCT03412877	II	Administration of Autologous T-Cells Genetically Engineered to Express T-Cell Receptors Reactive Against Mutated Neoantigens in People With Metastatic Cancer	270	Drug: Cyclophosphamide Drug: Fludarabine Drug: Aldesleukin Biological: Individual Patient TCR-Transduced PBL Drug: Pembrolizumab (KEYTRUDA)	Recruiting	National Institutes of Health Clinical Center (CC) (National Cancer Institute (NCI))
NCT04102436	II	A Phase II Study Using the Administration of Autologous T-Cells	210	Drug: Fludarabine	Recruiting	National Institutes of Health Clinical

		Engineered Using the Sleeping Beauty Transposon/Transposase System to Express T-Cell Receptors Reactive Against Mutated Neoantigens in Patients With Metastatic Cancer		Drug: Cyclophosphamide Drug: Aldesleukin Biological: Sleeping Beauty Transposed PBL		Center (CC) (National Cancer Institute (NCI))
NCT03552718	I	QUILT-2.025 NANT Neoepitope Yeast Vaccine (YE-NEO-001): Adjuvant Immunotherapy Using a Personalized Neoepitope Yeast-Based Vaccine To Induce T-Cell Responses In Subjects W/ Previously Treated Cancers.	16	Personalized recombinant yeast-based vaccine engineered to express multiple neoantigen epitopes (neoepitopes) based on an individual subject's tumor molecular profile.	Recruiting	NantBioScience, Inc.
NCT03970382	I	A Study of Gene Edited Autologous Neoantigen Targeted TCR T Cells With or Without Anti-PD-1 in Patients With Solid Tumors	148	Single dose of NeoTCR-P1 (targeting neoepitope on patient tumour) plus/minus nivolumab	Recruiting	PACT Pharma, Inc.

				480mg IV every four weeks for up to 6 doses.		

Accessing Multi-Omics Data

It is evident that there are a significant number of studies related to multi-omics techniques applied to breast cancer, though admittedly significantly fewer specifically related to immunotherapy. Such studies drive the need for a centralised resource to collate this data and provide it in an accessible format. DriverDBv3 is a multi-omics database for cancer driver gene research that can help to identify driving genes [99], whilst the Multi-Omics Breast Cancer Database (MOBCdb) is a breast cancer-specific database that integrates genomic, transcriptomic, and epigenomic data, along with clinical and drug response data of different breast cancer subtypes [100]. MOBCdb allows for the retrieval of gene expression, micro RNA (miRNA) expression, epigenetic information such as DNA methylation and drug response data and also provides an interface for simultaneous visualisation of multi-sample multi-omics data. Ultimately the database provides an accessible resource which enables researchers to identify novel potential biomarkers for targeted therapy and precision medicine [100]. A third database is ClinOmicsTrail[bc], which is a visual analytic tool for breast cancer that assesses existing drugs and potentially repurposed drugs, along with immunotherapeutic approaches [101]. This tool integrates clinical markers and (epi-)genomics and transcriptomic data to evaluate driver mutations, tumour mutational burden, and activity patterns of key cancer pathways, among other features [101].

In addition to these databases, there are a number of bioinformatic tools and platforms that facilitate the analysis of multi-omics data, such as bioCancer (https://bioconductor.org/packages/release/bioc/html/bioCancer.html), a package for Bioconductor, that visualises and interactively analyse multi-omics data. Different integration tools and methods for multi-omics data has recently been comprehensively reviewed by Subramanian and colleagues [102]. In general, the presence of these databases and analytical tools and methods greatly increase the accessibility of multi-omics data and will facilitate further research in the area.

Future Perspective

It is evident that the use of omics-type data, particularly integrated multi-omics analyses, has significant potential both from a patient stratification perspective and a therapeutic targeting perspective. The advances in bioinformatic and computational biology tools allow for more comprehensive and holistic analyses to be performed than was previously possible. Although these technologies allow for the identification of potentially novel therapies, when conducting clinical trials, it is important to ensure that appropriate, robust endpoints are used, and that studies or conclusions based on surrogate endpoints are interpreted with the appropriate caution. As time goes forward, it is anticipated that multi-omics approaches will become the standard, and integrate the 'classical' genomic, transcriptomic, and proteomic analyses with more specific areas which are showing increased importance such as miRNA screening, metabolomics, and omics-analysis of the role of dysbiosis in breast cancer. Systems biology, the subfield of computational biology which aims to construct accurate models of entire systems, is likely to play a significant role going forward for oncology as technologies improve, as accurate *in silico* simulation of important cancer pathways and superimposition of multi-omics-type data to these could be a bridge towards personalised therapy. It is anticipated that new and evolving therapies, including phototherapy, could be combined with the wealth of this 'omics-type' data to promote targeted and personalised applications, for instance by identifying patients who would best respond to particular combinatory therapies such as checkpoint blockade.

Executive Summary

<u>Basics of Breast Cancer:</u>

- Breast cancer remains one of the most common cancer types, and can be classified into four main molecular subtypes: luminal A, luminal B, HER2-enriched and triple-negative (ER-, PR-, HER2-).
- Breast cancer is staged at a clinical level according to the TNM classification of tumours which considers the tumour size, lymph node involvement, and metastases
- Breast cancer may currently be treated through radiotherapy, chemotherapy, and surgery. Although the current standard of care shows good therapeutic success, there is still unmet

need for some patients, particularly for triple-negative breast cancer, and there is a push for treatments that have less side-effects and are less invasive

Immunotherapy

- Immunotherapy is an approach that aims to utilise patients' immune systems to treat cancer, as oppose to the use of cytotoxic drugs or invasive surgery.
- There are numerous types of immunotherapy, such as immunotherapeutic vaccination and immune checkpoint blockade.
- Immune checkpoint blockade works on the principle that blocking the immune-downregulatory signals will help the immune system to remain active and target cancer cells, with common targets being CTLA-4 and PD-1/PD-L1
- Immunotherapy, particularly immune checkpoint blockade, holds significant potential and may allow cancer to become a more managed disease, as opposed to current treatments which generally do not target cancer specifically. However, there are improvements to be made due to the relatively low success rate, side-effects and toxicity of immunotherapies

Omics Data and Computational Biology

- The use of high-throughput data allows for tumour cell biology to be comprehensively analysed, and this can be performed at the mRNA (transcriptomics), protein (proteomics) or epigenome (epigenomics) level, among others
- In order to analyse such data effectively, it is essential to utilise bioinformatics and computational biology
- Computational biology also allows for the modelling of key cancer networks, but a step forward from this is multi-omics data analysis which can integrate information from the genome, to the transcriptome, to the proteome and beyond.
- Such integrated multi-omics analysis provides a deeper and more holistic view of cancer biology, and can consider not only what is occurring in the cancer cell but also its surrounding environment

Multi-Omics Data

- There are many uses of multi-omics data, such as comparing different patient cohorts to identify molecular signatures and understanding tumour mutational burden
- A popular approach in multi-omics data, which is linked to immunotherapy and has current clinical trials related to it, is neoantigen identification. Such approaches and the promise of training the immune system to target cancer, offer significant therapeutic promise
- To facilitate research into multi-omics data, there are a number of databases and resources such as MOBCdb and bioCancer that aid in the visualisation and analysis of the integrated data
- The use of multi-omics data may allow us to develop a deeper understanding of cancer cell biology between different patient cohorts, thus allowing for patient stratification. However, any clinical trial, particularly those investigating immunotherapy, should utilise robust endpoints where possible, due to the limited surrogacy of intermediate endpoints such as progression-free survival for overall survival

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 70(1), 7-30 (2020).
2. Meysami P, Rezaei F, Marashi SM, Amiri MM, Bakker E, Mokhtari-Azad T. Antitumor effects of a recombinant baculovirus displaying anti-HER2 scFv expressing Apoptin in HER2 positive SK-BR-3 breast cancer cells. *Future Virology* 14(3), 139-152 (2019).
3. Shayestehpour M, Moghim S, Salimi V *et al*. Selective replication of miR-145-regulated oncolytic adenovirus in MCF-7 breast cancer cells. *Future Virology* 11(10), 671-680 (2016).
4. Hoon Tan P, Ellis I, Allison K *et al*. The 2019 WHO classification of tumours of the breast. *Histopathology* doi:10.1111/his.14091 10.1111/his.14091 (2020).
5. Yersal O, Barutca S. Biological subtypes of breast cancer: Prognostic and therapeutic implications. *World J Clin Oncol* 5(3), 412-424 (2014).
6. Sharma GN, Dave R, Sanadya J, Sharma P, Sharma KK. Various types and management of breast cancer: an overview. *J Adv Pharm Technol Res* 1(2), 109-126 (2010).
7. Cancer, Breast https://www.ncbi.nlm.nih.gov/books/NBK482286/
8. Waks AG, Winer EP. Breast Cancer Treatment: A Review. *JAMA* 321(3), 288-300 (2019).
9. Li Y, Zhang X, Qiu J, Pang T, Huang L, Zeng Q. Comparisons of p53, KI67 and BRCA1 expressions in patients with different molecular subtypes of breast cancer and their relationships with pathology and prognosis. *Journal of B.U.ON. : official journal of the Balkan Union of Oncology* 24(6), 2361-2368 (2019).
10. Diagnostics Assessment Programme Tumour profiling tests to guide adjuvant chemotherapy decisions in people with breast cancer (update of DG10) Final scope https://www.nice.org.uk/guidance/dg34/documents/final-scope
11. Zhu X, Chen L, Huang B *et al*. The prognostic and predictive potential of Ki-67 in triple-negative breast cancer. *Sci Rep* 10(1), 225-225 (2020).
12. Bou-Dargham MJ, Liu Y, Sang QA, Zhang J. Subgrouping breast cancer patients based on immune evasion mechanisms unravels a high involvement of transforming growth factor-beta and decoy receptor 3. *PLoS One* 13(12), e0207799 (2018).
13. Brierley JD, Gospodarowicz MK, Wittekind C. *TNM Classification of Malignant Tumours*. (8th). John Wiley & Sons, Sussex, United Kingdom. (2016).
14. Giuliano AE, Edge SB, Hortobagyi GN. Eighth Edition of the AJCC Cancer Staging Manual: Breast Cancer. *Annals of surgical oncology* 25(7), 1783-1785 (2018).
15. Doll R. The Pierre Denoix Memorial Lecture: nature and nurture in the control of cancer. *European journal of cancer (Oxford, England : 1990)* 35(1), 16-23 (1999).
16. Greene FL, Sobin LH. A worldwide approach to the TNM staging system: collaborative efforts of the AJCC and UICC. *Journal of surgical oncology* 99(5), 269-272 (2009).
17. Cserni G, Chmielik E, Cserni B, Tot T. The new TNM-based staging of breast cancer. *Virchows Archiv : an international journal of pathology* 472(5), 697-703 (2018).
18. Early and locally advanced breast cancer: diagnosis and management https://www.nice.org.uk/guidance/ng101 (16th February).
19. Yoon EC, Schwartz C, Brogi E, Ventura K, Wen H, Darvishian F. Impact of biomarkers and genetic profiling on breast cancer prognostication: A comparative analysis of the 8th edition of breast cancer staging system. *The breast journal* 25(5), 829-837 (2019).
20. Irwin GW, Bannon F, Coles CE *et al*. The NeST (neoadjuvant systemic therapy in breast cancer) study – Protocol for a prospective multi-centre cohort study to assess the current utilization and short-term outcomes of neoadjuvant systemic therapies in breast cancer. *International Journal of Surgery Protocols* 18 5-11 (2019).
21. Levasseur N, Willemsma KA, Li H *et al*. Efficacy of Neoadjuvant Endocrine Therapy Versus Neoadjuvant Chemotherapy in ER-positive Breast Cancer: Results From a Prospective Institutional Database. *Clinical breast cancer* 19(6), e683-e689 (2019).

22. Asselain B, Barlow W, Bartlett J *et al*. Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials. *The Lancet Oncology* 19(1), 27-39 (2018).
23. Gustavo Werutsky G, Untch M, Hanusch C *et al*. Locoregional recurrence risk after neoadjuvant chemotherapy: A pooled analysis of nine prospective neoadjuvant breast cancer trials. *European journal of cancer (Oxford, England : 1990)* 130 92-101 (2020).
24. Han R, Regpala S, Slodkowska E *et al*. Lack of Standardization in the Processing and Reporting of Post-Neoadjuvant Breast Cancer Specimens: A Survey of Canadian Pathologists and Pathology Assistants. *Archives of pathology & laboratory medicine* doi:10.5858/arpa.2019-0539-OA (2020).
25. Hwang ES, Lichtensztajn DY, Gomez SL, Fowble B, Clarke CA. Survival after lumpectomy and mastectomy for early stage invasive breast cancer: the effect of age and hormone receptor status. *Cancer* 119(7), 1402-1411 (2013).
26. Advanced breast cancer: diagnosis and treatment https://www.nice.org.uk/guidance/cg81 (16th February).
27. Li X, Liu Y, Fu F *et al*. Single NIR Laser-Activated Multifunctional Nanoparticles for Cascaded Photothermal and Oxygen-Independent Photodynamic Therapy. *Nano-Micro Letters* 11(1), 68 (2019).
28. Yi G, Hong SH, Son J *et al*. Recent advances in nanoparticle carriers for photodynamic therapy. *Quant Imaging Med Surg* 8(4), 433-443 (2018).
29. Liu Y, Bhattarai P, Dai Z, Chen X. Photothermal therapy and photoacoustic imaging via nanotheranostics in fighting cancer. *Chemical Society reviews* 48(7), 2053-2108 (2019).
30. Liang X, Ye X, Wang C *et al*. Photothermal cancer immunotherapy by erythrocyte membrane-coated black phosphorus formulation. *Journal of controlled release : official journal of the Controlled Release Society* 296 150-161 (2019).
31. Lee J, Ahn E, Kissick HT, Ahmed R. Reinvigorating Exhausted T Cells by Blockade of the PD-1 Pathway. *Forum on immunopathological diseases and therapeutics* 6(1-2), 7-17 (2015).
32. Allahverdiyev A, Tari G, Bagirova M, Abamor ES. Current Approaches in Development of Immunotherapeutic Vaccines for Breast Cancer. *Journal of breast cancer* 21(4), 343-353 (2018).
33. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature* 480(7378), 480-489 (2011).
34. Grégoire M. What's the place of immunotherapy in malignant mesothelioma treatments? *Cell Adh Migr* 4(1), 153-161 (2010).
35. Abbott M, Ustoyev Y. Cancer and the Immune System: The History and Background of Immunotherapy. *Semin Oncol Nurs* 35(5), 150923-150923 (2019).
36. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* 168(4), 707-723 (2017).
37. Yu L-Y, Tang J, Zhang C-M *et al*. New Immunotherapy Strategies in Breast Cancer. *Int J Environ Res Public Health* 14(1), 68 (2017).
38. Migali C, Milano M, Trapani D *et al*. Strategies to modulate the immune system in breast cancer: checkpoint inhibitors and beyond. *Ther Adv Med Oncol* 8(5), 360-374 (2016).
39. Guo C, Manjili MH, Subjeck JR, Sarkar D, Fisher PB, Wang X-Y. Therapeutic cancer vaccines: past, present, and future. *Adv Cancer Res* 119 421-475 (2013).
40. Miles D, Papazisis K. Rationale for the clinical development of STn-KLH (Theratope) and anti-MUC-1 vaccines in breast cancer. *Clinical breast cancer* 3 Suppl 4 S134-S138 (2003).
41. Rakha EA, Boyce RWG, Abd El-Rehim D *et al*. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod Pathol* 18(10), 1295-1304 (2005).
42. Ladjemi MZ, Jacot W, Chardès T, Pèlegrin A, Navarro-Teulon I. Anti-HER2 vaccines: new prospects for breast cancer therapy. *Cancer Immunol Immunother* 59(9), 1295-1312 (2010).

43. Incorvati JA, Shah S, Mu Y, Lu J. Targeted therapy for HER2 positive breast cancer. *Journal of Hematology & Oncology* 6(1), 38 (2013).
44. Al-Awadhi A, Lee Murray J, Ibrahim NK. Developing anti-HER2 vaccines: Breast cancer experience. *Int J Cancer* 143(9), 2126-2132 (2018).
45. Disis ML, Wallace DR, Gooley TA *et al*. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. *J Clin Oncol* 27(28), 4685-4692 (2009).
46. Blackwell KL, Burstein HJ, Storniolo AM *et al*. Overall survival benefit with lapatinib in combination with trastuzumab for patients with human epidermal growth factor receptor 2-positive metastatic breast cancer: final results from the EGF104900 Study. *J Clin Oncol* 30(21), 2585-2592 (2012).
47. Murray JL, Gillogly ME, Przepiorka D *et al*. Toxicity, immunogenicity, and induction of E75-specific tumor-lytic CTLs by HER-2 peptide E75 (369-377) combined with granulocyte macrophage colony-stimulating factor in HLA-A2+ patients with metastatic breast and ovarian cancer. *Clin Cancer Res* 8(11), 3407-3418 (2002).
48. Mittendorf EA, Clifton GT, Holmes JP *et al*. Final report of the phase I/II clinical trial of the E75 (nelipepimut-S) vaccine with booster inoculations to prevent disease recurrence in high-risk breast cancer patients. *Ann Oncol* 25(9), 1735-1742 (2014).
49. Mittendorf EA, Ardavanis A, Litton JK *et al*. Primary analysis of a prospective, randomized, single-blinded phase II trial evaluating the HER2 peptide GP2 vaccine in breast cancer patients to prevent recurrence. *Oncotarget* 7(40), 66192-66201 (2016).
50. Mittendorf EA, Ardavanis A, Symanowski J *et al*. Primary analysis of a prospective, randomized, single-blinded phase II trial evaluating the HER2 peptide AE37 vaccine in breast cancer patients to prevent recurrence. *Ann Oncol* 27(7), 1241-1248 (2016).
51. Ibrahim NK, Murray JL, Zhou D *et al*. Survival Advantage in Patients with Metastatic Breast Cancer Receiving Endocrine Therapy plus Sialyl Tn-KLH Vaccine: Post Hoc Analysis of a Large Randomized Trial. *J Cancer* 4(7), 577-584 (2013).
52. Mohebtash M, Tsang K-Y, Madan RA *et al*. A pilot study of MUC-1/CEA/TRICOM poxviral-based vaccine in patients with metastatic breast and ovarian cancer. *Clin Cancer Res* 17(22), 7164-7173 (2011).
53. Park JW, Melisko ME, Esserman LJ, Jones LA, Wollan JB, Sims R. Treatment with autologous antigen-presenting cells activated with the HER-2 based antigen Lapuleucel-T: results of a phase I study in immunologic and clinical activity in HER-2 overexpressing breast cancer. *J Clin Oncol* 25(24), 3680-3687 (2007).
54. Brossart P, Wirths S, Stuhler G, Reichardt VL, Kanz L, Brugger W. Induction of cytotoxic T-lymphocyte responses in vivo after vaccinations with peptide-pulsed dendritic cells. *Blood* 96(9), 3102-3108 (2000).
55. Czerniecki BJ, Koski GK, Koldovsky U *et al*. Targeting HER-2/neu in early breast cancer development using dendritic cells with staged interleukin-12 burst secretion. *Cancer Res* 67(4), 1842-1852 (2007).
56. Solinas C, Aiello M, Migliori E, Willard-Gallo K, Emens LA. Breast cancer vaccines: Heeding the lessons of the past to guide a path forward. *Cancer Treat Rev* 84 101947-101947 (2019).
57. Wagner S, Mullins CS, Linnebacher M. Colorectal cancer vaccines: Tumor-associated antigens vs neoantigens. *World J Gastroenterol* 24(48), 5418-5432 (2018).
58. Vonderheide RH, Lorusso PM, Khalil M *et al*. Tremelimumab in combination with exemestane in patients with advanced breast cancer and treatment-associated modulation of inducible costimulator expression on patient T cells. *Clin Cancer Res* 16(13), 3485-3494 (2010).
59. Diab A, Mcarthur HL, Solomon SB *et al*. A pilot study of preoperative (Pre-op), single-dose ipilimumab (Ipi) and/or cryoablation (Cryo) in women (pts) with early-stage/resectable breast cancer (ESBC). *Journal of Clinical Oncology* 32(15_suppl), 1098-1098 (2014).

60. Brignone C, Gutierrez M, Mefti F *et al*. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. *J Transl Med* 8 71-71 (2010).
61. Nanda R, Chow LQM, Dees EC *et al*. Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. *J Clin Oncol* 34(21), 2460-2467 (2016).
62. Adams S, Diamond J, Hamilton E *et al*. Abstract P2-11-06: Safety and clinical activity of atezolizumab (anti-PDL1) in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer. *Cancer Res* 76(4 Supplement), P2-11-06 (2016).
63. Tan A, Porcher R, Crequit P, Ravaud P, Dechartres A. Differences in Treatment Effect Size Between Overall Survival and Progression-Free Survival in Immunotherapy Trials: A Meta-Epidemiologic Study of Trials With Results Posted at ClinicalTrials.gov. *Journal of Clinical Oncology* 35(15), 1686-1694 (2017).
64. Burzykowski T, Buyse M, Piccart-Gebhart MJ *et al*. Evaluation of Tumor Response, Disease Control, Progression-Free Survival, and Time to Progression As Potential Surrogate End Points in Metastatic Breast Cancer. *Journal of Clinical Oncology* 26(12), 1987-1992 (2008).
65. Michiels S, Pugliano L, Marguet S *et al*. Progression-free survival as surrogate end point for overall survival in clinical trials of HER2-targeted agents in HER2-positive metastatic breast cancer. *Ann Oncol* 27(6), 1029-1034 (2016).
66. Thapa B, Walkiewicz M, Rivalland G *et al*. Immune microenvironment in mesothelioma: Looking beyond PD-L1. *Journal of Clinical Oncology* 35(15_suppl), 8515-8515 (2017).
67. Tian K, Rajendran R, Doddananjaiah M, Krstic-Demonacos M, Schwartz J-M. Dynamics of DNA damage induced pathways to cancer. *PLoS One* 8(9), e72303-e72303 (2013).
68. Bakker E, Tian K, Mutti L, Demonacos C, Schwartz J-M, Krstic-Demonacos M. Insight into glucocorticoid receptor signalling through interactome model analysis. *PLOS Computational Biology* 13(11), e1005825 (2017).
69. Tian K, Bakker E, Hussain M *et al*. p53 modeling as a route to mesothelioma patients stratification and novel therapeutic identification. *J Transl Med* 16(1), 282 (2018).
70. Bueno R, Stawiski EW, Goldstein LD *et al*. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. *Nature Genetics* 48(4), 407-416 (2016).
71. Chakraborty S, Hosen MI, Ahmed M, Shekhar HU. Onco-Multi-OMICS Approach: A New Frontier in Cancer Research. *Biomed Res Int* 2018 9836256-9836256 (2018).
72. Tyanova S, Albrechtsen R, Kronqvist P, Cox J, Mann M, Geiger T. Proteomic maps of breast cancer subtypes. *Nat Commun* 7 10259-10259 (2016).
73. Mertins P, Mani DR, Ruggles KV *et al*. Proteogenomics connects somatic mutations to signalling in breast cancer. *Nature* 534(7605), 55-62 (2016).
74. Terunuma A, Putluri N, Mishra P *et al*. MYC-driven accumulation of 2-hydroxyglutarate is associated with breast cancer prognosis. *J Clin Invest* 124(1), 398-412 (2014).
75. Auslander N, Yizhak K, Weinstock A *et al*. A joint analysis of transcriptomic and metabolomic data uncovers enhanced enzyme-metabolite coupling in breast cancer. *Sci Rep* 6 29662-29662 (2016).
76. Huang H, Cao S, Zhang Z, Li L, Chen F, Wu Q. Multiple omics analysis of the protective effects of SFN on estrogen-dependent breast cancer cells. *Molecular Biology Reports* 47(5), 3331-3346 (2020).
77. Wang J, Li S, Lin S *et al*. B-cell lymphoma 2 family genes show a molecular pattern of spatiotemporal heterogeneity in gynaecologic and breast cancer. *Cell Prolif* doi:10.1111/cpr.12826 e12826 (2020).
78. Karim MA, Samad A, Adhikari UK *et al*. A Multi-Omics Analysis of Bone Morphogenetic Protein 5 (BMP5) mRNA Expression and Clinical Prognostic Outcomes in Different Cancers Using Bioinformatics Approaches. *Biomedicines* 8(2), (2020).

79. Rhodes DR, Yu J, Shanker K *et al*. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* 6(1), 1-6 (2004).
80. Shin G, Kang TW, Yang S, Baek SJ, Jeong YS, Kim SY. GENT: gene expression database of normal and tumor tissues. *Cancer Inform* 10 149-157 (2011).
81. Chandrashekar DS, Bashel B, Balasubramanya SaH *et al*. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia* 19(8), 649-658 (2017).
82. Mizuno H, Kitada K, Nakai K, Sarai A. PrognoScan: a new database for meta-analysis of the prognostic value of genes. *BMC medical genomics* 2 18-18 (2009).
83. Cerami E, Gao J, Dogrusoz U *et al*. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2(5), 401-404 (2012).
84. Gao J, Aksoy BA, Dogrusoz U *et al*. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 6(269), pl1 (2013).
85. Cui H, Kong H, Peng F *et al*. Inferences of Individual Drug Response-Related Long Non-coding RNAs Based on Integrating Multi-omics Data in Breast Cancer. *Mol Ther Nucleic Acids* 20 128-139 (2020).
86. Finotello F, Eduati F. Multi-Omics Profiling of the Tumor Microenvironment: Paving the Way to Precision Immuno-Oncology. 8(430), (2018).
87. Soysal SD, Tzankov A, Muenst SE. Role of the Tumor Microenvironment in Breast Cancer. *Pathobiology* 82(3-4), 142-152 (2015).
88. Xiao Y, Ma D, Zhao S *et al*. Multi-Omics Profiling Reveals Distinct Microenvironment Characterization and Suggests Immune Escape Mechanisms of Triple-Negative Breast Cancer. *Clinical Cancer Research* 25(16), 5002 (2019).
89. Mcgrail DJ, Federico L, Li Y *et al*. Multi-omics analysis reveals neoantigen-independent immune cell infiltration in copy-number driven cancers. *Nat Commun* 9(1), 1317-1317 (2018).
90. Kan Z, Ding Y, Kim J *et al*. Multi-omics profiling of younger Asian breast cancers reveals distinctive molecular signatures. *Nat Commun* 9(1), 1725-1725 (2018).
91. Buisseret L, Garaud S, De Wind A *et al*. Tumor-infiltrating lymphocyte composition, organization and PD-1/ PD-L1 expression are linked in breast cancer. *Oncoimmunology* 6(1), e1257452 (2017).
92. Barroso-Sousa R, Jain E, Cohen O *et al*. Prevalence and mutational determinants of high tumor mutation burden in breast cancer. *Annals of Oncology* 31(3), 387-394 (2020).
93. Chen Z, Wen W, Bao J *et al*. Integrative genomic analyses of APOBEC-mutational signature, expression and germline deletion of APOBEC3 genes, and immunogenicity in multiple cancer types. *BMC Medical Genomics* 12(1), 131 (2019).
94. Burns MB, Lackey L, Carpenter MA *et al*. APOBEC3B is an enzymatic source of mutation in breast cancer. *Nature* 494(7437), 366-370 (2013).
95. Richters MM, Xia H, Campbell KM, Gillanders WE, Griffith OL, Griffith M. Best practices for bioinformatic characterization of neoantigens for clinical utility. *Genome Medicine* 11(1), 56 (2019).
96. Rizvi NA, Hellmann MD, Snyder A *et al*. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 348(6230), 124-128 (2015).
97. Van Allen EM, Miao D, Schilling B *et al*. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 350(6257), 207-211 (2015).
98. Peng M, Mo Y, Wang Y *et al*. Neoantigen vaccine: an emerging tumor immunotherapy. *Molecular Cancer* 18(1), 128 (2019).
99. Liu S-H, Shen P-C, Chen C-Y *et al*. DriverDBv3: a multi-omics database for cancer driver gene research. *Nucleic Acids Research* 48(D1), D863-D870 (2019).

100. Xie B, Yuan Z, Yang Y, Sun Z, Zhou S, Fang X. MOBCdb: a comprehensive database integrating multi-omics data on breast cancer for precision medicine. *Breast Cancer Res Treat* 169(3), 625-632 (2018).
101. Schneider L, Kehl T, Thedinga K *et al*. ClinOmicsTrailbc: a visual analytics tool for breast cancer treatment stratification. *Bioinformatics* 35(24), 5171-5181 (2019).
102. Subramanian I, Verma S, Kumar S, Jere A, Anamika K. Multi-omics Data Integration, Interpretation, and Its Application. *Bioinform Biol Insights* 14 1177932219899051-1177932219899051 (2020).

References With Annotations (After EndNote Update When Track Changes Accepted)

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 70(1), 7-30 (2020).
2. Meysami P, Rezaei F, Marashi SM, Amiri MM, Bakker E, Mokhtari-Azad T. Antitumor effects of a recombinant baculovirus displaying anti-HER2 scFv expressing Apoptin in HER2 positive SK-BR-3 breast cancer cells. *Future Virology* 14(3), 139-152 (2019).
3. Shayestehpour M, Moghim S, Salimi V *et al*. Selective replication of miR-145-regulated oncolytic adenovirus in MCF-7 breast cancer cells. *Future Virology* 11(10), 671-680 (2016).
4. Hoon Tan P, Ellis I, Allison K *et al*. The 2019 WHO classification of tumours of the breast. *Histopathology* doi:10.1111/his.14091 10.1111/his.14091 (2020).
5. Yersal O, Barutca S. Biological subtypes of breast cancer: Prognostic and therapeutic implications. *World J Clin Oncol* 5(3), 412-424 (2014).
6. Sharma GN, Dave R, Sanadya J, Sharma P, Sharma KK. Various types and management of breast cancer: an overview. *J Adv Pharm Technol Res* 1(2), 109-126 (2010).
7. Cancer, Breast https://www.ncbi.nlm.nih.gov/books/NBK482286/
8. Waks AG, Winer EP. Breast Cancer Treatment: A Review. *JAMA* 321(3), 288-300 (2019).
9. Li Y, Zhang X, Qiu J, Pang T, Huang L, Zeng Q. Comparisons of p53, KI67 and BRCA1 expressions in patients with different molecular subtypes of breast cancer and their relationships with pathology and prognosis. *Journal of B.U.ON. : official journal of the Balkan Union of Oncology* 24(6), 2361-2368 (2019).
10. Diagnostics Assessment Programme Tumour profiling tests to guide adjuvant chemotherapy decisions in people with breast cancer (update of DG10) Final scope https://www.nice.org.uk/guidance/dg34/documents/final-scope
11. Zhu X, Chen L, Huang B *et al*. The prognostic and predictive potential of Ki-67 in triple-negative breast cancer. *Sci Rep* 10(1), 225-225 (2020).
12. Bou-Dargham MJ, Liu Y, Sang QA, Zhang J. Subgrouping breast cancer patients based on immune evasion mechanisms unravels a high involvement of transforming growth factor-beta and decoy receptor 3. *PLoS One* 13(12), e0207799 (2018).
13. Brierley JD, Gospodarowicz MK, Wittekind C. *TNM Classification of Malignant Tumours*. (8th). John Wiley & Sons, Sussex, United Kingdom. (2016).
14. Giuliano AE, Edge SB, Hortobagyi GN. Eighth Edition of the AJCC Cancer Staging Manual: Breast Cancer. *Annals of surgical oncology* 25(7), 1783-1785 (2018).
15. Doll R. The Pierre Denoix Memorial Lecture: nature and nurture in the control of cancer. *European journal of cancer (Oxford, England : 1990)* 35(1), 16-23 (1999).
16. Greene FL, Sobin LH. A worldwide approach to the TNM staging system: collaborative efforts of the AJCC and UICC. *Journal of surgical oncology* 99(5), 269-272 (2009).
17. Cserni G, Chmielik E, Cserni B, Tot T. The new TNM-based staging of breast cancer. *Virchows Archiv : an international journal of pathology* 472(5), 697-703 (2018).
18. Early and locally advanced breast cancer: diagnosis and management https://www.nice.org.uk/guidance/ng101 (16th February).
19. Yoon EC, Schwartz C, Brogi E, Ventura K, Wen H, Darvishian F. Impact of biomarkers and genetic profiling on breast cancer prognostication: A comparative analysis of the 8th edition of breast cancer staging system. *The breast journal* 25(5), 829-837 (2019).
20. Irwin GW, Bannon F, Coles CE *et al*. The NeST (neoadjuvant systemic therapy in breast cancer) study – Protocol for a prospective multi-centre cohort study to assess the current utilization and short-term outcomes of neoadjuvant systemic therapies in breast cancer. *International Journal of Surgery Protocols* 18 5-11 (2019).

21. Levasseur N, Willemsma KA, Li H *et al*. Efficacy of Neoadjuvant Endocrine Therapy Versus Neoadjuvant Chemotherapy in ER-positive Breast Cancer: Results From a Prospective Institutional Database. *Clinical breast cancer* 19(6), e683-e689 (2019).
22. Asselain B, Barlow W, Bartlett J *et al*. Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials. *The Lancet Oncology* 19(1), 27-39 (2018).
23. Gustavo Werutsky G, Untch M, Hanusch C *et al*. Locoregional recurrence risk after neoadjuvant chemotherapy: A pooled analysis of nine prospective neoadjuvant breast cancer trials. *European journal of cancer (Oxford, England : 1990)* 130 92-101 (2020).
24. Han R, Regpala S, Slodkowska E *et al*. Lack of Standardization in the Processing and Reporting of Post-Neoadjuvant Breast Cancer Specimens: A Survey of Canadian Pathologists and Pathology Assistants. *Archives of pathology & laboratory medicine* doi:10.5858/arpa.2019-0539-OA (2020).
25. Hwang ES, Lichtensztajn DY, Gomez SL, Fowble B, Clarke CA. Survival after lumpectomy and mastectomy for early stage invasive breast cancer: the effect of age and hormone receptor status. *Cancer* 119(7), 1402-1411 (2013).
26. Advanced breast cancer: diagnosis and treatment https://www.nice.org.uk/guidance/cg81 (16th February).
27. Li X, Liu Y, Fu F *et al*. Single NIR Laser-Activated Multifunctional Nanoparticles for Cascaded Photothermal and Oxygen-Independent Photodynamic Therapy. *Nano-Micro Letters* 11(1), 68 (2019).
28. Yi G, Hong SH, Son J *et al*. Recent advances in nanoparticle carriers for photodynamic therapy. *Quant Imaging Med Surg* 8(4), 433-443 (2018).
29. Liu Y, Bhattarai P, Dai Z, Chen X. Photothermal therapy and photoacoustic imaging via nanotheranostics in fighting cancer. *Chemical Society reviews* 48(7), 2053-2108 (2019).
30. Liang X, Ye X, Wang C *et al*. Photothermal cancer immunotherapy by erythrocyte membrane-coated black phosphorus formulation. *Journal of controlled release : official journal of the Controlled Release Society* 296 150-161 (2019).
31. Lee J, Ahn E, Kissick HT, Ahmed R. Reinvigorating Exhausted T Cells by Blockade of the PD-1 Pathway. *Forum on immunopathological diseases and therapeutics* 6(1-2), 7-17 (2015).
32. Allahverdiyev A, Tari G, Bagirova M, Abamor ES. Current Approaches in Development of Immunotherapeutic Vaccines for Breast Cancer. *Journal of breast cancer* 21(4), 343-353 (2018).
33. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature* 480(7378), 480-489 (2011).
34. Grégoire M. What's the place of immunotherapy in malignant mesothelioma treatments? *Cell Adh Migr* 4(1), 153-161 (2010).
35. Abbott M, Ustoyev Y. Cancer and the Immune System: The History and Background of Immunotherapy. *Semin Oncol Nurs* 35(5), 150923-150923 (2019).
36. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* 168(4), 707-723 (2017).
37. Yu L-Y, Tang J, Zhang C-M *et al*. New Immunotherapy Strategies in Breast Cancer. *Int J Environ Res Public Health* 14(1), 68 (2017).
38. Migali C, Milano M, Trapani D *et al*. Strategies to modulate the immune system in breast cancer: checkpoint inhibitors and beyond. *Ther Adv Med Oncol* 8(5), 360-374 (2016).
39. Guo C, Manjili MH, Subjeck JR, Sarkar D, Fisher PB, Wang X-Y. Therapeutic cancer vaccines: past, present, and future. *Adv Cancer Res* 119 421-475 (2013).
40. Miles D, Papazisis K. Rationale for the clinical development of STn-KLH (Theratope) and anti-MUC-1 vaccines in breast cancer. *Clinical breast cancer* 3 Suppl 4 S134-S138 (2003).

41. Rakha EA, Boyce RWG, Abd El-Rehim D *et al*. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod Pathol* 18(10), 1295-1304 (2005).
42. Ladjemi MZ, Jacot W, Chardès T, Pèlegrin A, Navarro-Teulon I. Anti-HER2 vaccines: new prospects for breast cancer therapy. *Cancer Immunol Immunother* 59(9), 1295-1312 (2010).
43. Incorvati JA, Shah S, Mu Y, Lu J. Targeted therapy for HER2 positive breast cancer. *Journal of Hematology & Oncology* 6(1), 38 (2013).
44. Al-Awadhi A, Lee Murray J, Ibrahim NK. Developing anti-HER2 vaccines: Breast cancer experience. *Int J Cancer* 143(9), 2126-2132 (2018).
45. Disis ML, Wallace DR, Gooley TA *et al*. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. *J Clin Oncol* 27(28), 4685-4692 (2009).
46. Blackwell KL, Burstein HJ, Storniolo AM *et al*. Overall survival benefit with lapatinib in combination with trastuzumab for patients with human epidermal growth factor receptor 2-positive metastatic breast cancer: final results from the EGF104900 Study. *J Clin Oncol* 30(21), 2585-2592 (2012).
47. Murray JL, Gillogly ME, Przepiorka D *et al*. Toxicity, immunogenicity, and induction of E75-specific tumor-lytic CTLs by HER-2 peptide E75 (369-377) combined with granulocyte macrophage colony-stimulating factor in HLA-A2+ patients with metastatic breast and ovarian cancer. *Clin Cancer Res* 8(11), 3407-3418 (2002).
48. Mittendorf EA, Clifton GT, Holmes JP *et al*. Final report of the phase I/II clinical trial of the E75 (nelipepimut-S) vaccine with booster inoculations to prevent disease recurrence in high-risk breast cancer patients. *Ann Oncol* 25(9), 1735-1742 (2014).
49. Mittendorf EA, Ardavanis A, Litton JK *et al*. Primary analysis of a prospective, randomized, single-blinded phase II trial evaluating the HER2 peptide GP2 vaccine in breast cancer patients to prevent recurrence. *Oncotarget* 7(40), 66192-66201 (2016).
50. Mittendorf EA, Ardavanis A, Symanowski J *et al*. Primary analysis of a prospective, randomized, single-blinded phase II trial evaluating the HER2 peptide AE37 vaccine in breast cancer patients to prevent recurrence. *Ann Oncol* 27(7), 1241-1248 (2016).
51. Ibrahim NK, Murray JL, Zhou D *et al*. Survival Advantage in Patients with Metastatic Breast Cancer Receiving Endocrine Therapy plus Sialyl Tn-KLH Vaccine: Post Hoc Analysis of a Large Randomized Trial. *J Cancer* 4(7), 577-584 (2013).
52. Mohebtash M, Tsang K-Y, Madan RA *et al*. A pilot study of MUC-1/CEA/TRICOM poxviral-based vaccine in patients with metastatic breast and ovarian cancer. *Clin Cancer Res* 17(22), 7164-7173 (2011).
53. Park JW, Melisko ME, Esserman LJ, Jones LA, Wollan JB, Sims R. Treatment with autologous antigen-presenting cells activated with the HER-2 based antigen Lapuleucel-T: results of a phase I study in immunologic and clinical activity in HER-2 overexpressing breast cancer. *J Clin Oncol* 25(24), 3680-3687 (2007).
54. Brossart P, Wirths S, Stuhler G, Reichardt VL, Kanz L, Brugger W. Induction of cytotoxic T-lymphocyte responses in vivo after vaccinations with peptide-pulsed dendritic cells. *Blood* 96(9), 3102-3108 (2000).
55. Czerniecki BJ, Koski GK, Koldovsky U *et al*. Targeting HER-2/neu in early breast cancer development using dendritic cells with staged interleukin-12 burst secretion. *Cancer Res* 67(4), 1842-1852 (2007).
56. Solinas C, Aiello M, Migliori E, Willard-Gallo K, Emens LA. Breast cancer vaccines: Heeding the lessons of the past to guide a path forward. *Cancer Treat Rev* 84 101947-101947 (2019).
57. Wagner S, Mullins CS, Linnebacher M. Colorectal cancer vaccines: Tumor-associated antigens vs neoantigens. *World J Gastroenterol* 24(48), 5418-5432 (2018).
58. Vonderheide RH, Lorusso PM, Khalil M *et al*. Tremelimumab in combination with exemestane in patients with advanced breast cancer and treatment-associated modulation

of inducible costimulator expression on patient T cells. *Clin Cancer Res* 16(13), 3485-3494 (2010).
59. Diab A, Mcarthur HL, Solomon SB *et al*. A pilot study of preoperative (Pre-op), single-dose ipilimumab (Ipi) and/or cryoablation (Cryo) in women (pts) with early-stage/resectable breast cancer (ESBC). *Journal of Clinical Oncology* 32(15_suppl), 1098-1098 (2014).
60. Brignone C, Gutierrez M, Mefti F *et al*. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. *J Transl Med* 8 71-71 (2010).
61. Nanda R, Chow LQM, Dees EC *et al*. Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. *J Clin Oncol* 34(21), 2460-2467 (2016).
62. Adams S, Diamond J, Hamilton E *et al*. Abstract P2-11-06: Safety and clinical activity of atezolizumab (anti-PDL1) in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer. *Cancer Res* 76(4 Supplement), P2-11-06 (2016).
63. Tan A, Porcher R, Crequit P, Ravaud P, Dechartres A. Differences in Treatment Effect Size Between Overall Survival and Progression-Free Survival in Immunotherapy Trials: A Meta-Epidemiologic Study of Trials With Results Posted at ClinicalTrials.gov. *Journal of Clinical Oncology* 35(15), 1686-1694 (2017).

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64. Burzykowski T, Buyse M, Piccart-Gebhart MJ *et al*. Evaluation of Tumor Response, Disease Control, Progression-Free Survival, and Time to Progression As Potential Surrogate End Points in Metastatic Breast Cancer. *Journal of Clinical Oncology* 26(12), 1987-1992 (2008).
65. Michiels S, Pugliano L, Marguet S *et al*. Progression-free survival as surrogate end point for overall survival in clinical trials of HER2-targeted agents in HER2-positive metastatic breast cancer. *Ann Oncol* 27(6), 1029-1034 (2016).
66. Thapa B, Walkiewicz M, Rivalland G *et al*. Immune microenvironment in mesothelioma: Looking beyond PD-L1. *Journal of Clinical Oncology* 35(15_suppl), 8515-8515 (2017).
67. Tian K, Rajendran R, Doddananjaiah M, Krstic-Demonacos M, Schwartz J-M. Dynamics of DNA damage induced pathways to cancer. *PLoS One* 8(9), e72303-e72303 (2013).
68. Bakker E, Tian K, Mutti L, Demonacos C, Schwartz J-M, Krstic-Demonacos M. Insight into glucocorticoid receptor signalling through interactome model analysis. *PLOS Computational Biology* 13(11), e1005825 (2017).
69. Tian K, Bakker E, Hussain M *et al*. p53 modeling as a route to mesothelioma patients stratification and novel therapeutic identification. *J Transl Med* 16(1), 282 (2018).
70. Bueno R, Stawiski EW, Goldstein LD *et al*. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. *Nature Genetics* 48(4), 407-416 (2016).
71. Chakraborty S, Hosen MI, Ahmed M, Shekhar HU. Onco-Multi-OMICS Approach: A New Frontier in Cancer Research. *Biomed Res Int* 2018 9836256-9836256 (2018).
72. Tyanova S, Albrechtsen R, Kronqvist P, Cox J, Mann M, Geiger T. Proteomic maps of breast cancer subtypes. *Nat Commun* 7 10259-10259 (2016).
73. Mertins P, Mani DR, Ruggles KV *et al*. Proteogenomics connects somatic mutations to signalling in breast cancer. *Nature* 534(7605), 55-62 (2016).
74. Terunuma A, Putluri N, Mishra P *et al*. MYC-driven accumulation of 2-hydroxyglutarate is associated with breast cancer prognosis. *J Clin Invest* 124(1), 398-412 (2014).
75. Auslander N, Yizhak K, Weinstock A *et al*. A joint analysis of transcriptomic and metabolomic data uncovers enhanced enzyme-metabolite coupling in breast cancer. *Sci Rep* 6 29662-29662 (2016).

76. Huang H, Cao S, Zhang Z, Li L, Chen F, Wu Q. Multiple omics analysis of the protective effects of SFN on estrogen-dependent breast cancer cells. *Molecular Biology Reports* 47(5), 3331-3346 (2020).
77. Wang J, Li S, Lin S *et al*. B-cell lymphoma 2 family genes show a molecular pattern of spatiotemporal heterogeneity in gynaecologic and breast cancer. *Cell Prolif* doi:10.1111/cpr.12826 e12826 (2020).
78. Karim MA, Samad A, Adhikari UK *et al*. A Multi-Omics Analysis of Bone Morphogenetic Protein 5 (BMP5) mRNA Expression and Clinical Prognostic Outcomes in Different Cancers Using Bioinformatics Approaches. *Biomedicines* 8(2), (2020).
79. Rhodes DR, Yu J, Shanker K *et al*. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* 6(1), 1-6 (2004).
80. Shin G, Kang TW, Yang S, Baek SJ, Jeong YS, Kim SY. GENT: gene expression database of normal and tumor tissues. *Cancer Inform* 10 149-157 (2011).
81. Chandrashekar DS, Bashel B, Balasubramanya SaH *et al*. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia* 19(8), 649-658 (2017).
82. Mizuno H, Kitada K, Nakai K, Sarai A. PrognoScan: a new database for meta-analysis of the prognostic value of genes. *BMC medical genomics* 2 18-18 (2009).
83. Cerami E, Gao J, Dogrusoz U *et al*. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2(5), 401-404 (2012).
84. Gao J, Aksoy BA, Dogrusoz U *et al*. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 6(269), pl1 (2013).
85. Cui H, Kong H, Peng F *et al*. Inferences of Individual Drug Response-Related Long Non-coding RNAs Based on Integrating Multi-omics Data in Breast Cancer. *Mol Ther Nucleic Acids* 20 128-139 (2020).
86. Finotello F, Eduati F. Multi-Omics Profiling of the Tumor Microenvironment: Paving the Way to Precision Immuno-Oncology. 8(430), (2018).
87. Soysal SD, Tzankov A, Muenst SE. Role of the Tumor Microenvironment in Breast Cancer. *Pathobiology* 82(3-4), 142-152 (2015).
88. Xiao Y, Ma D, Zhao S *et al*. Multi-Omics Profiling Reveals Distinct Microenvironment Characterization and Suggests Immune Escape Mechanisms of Triple-Negative Breast Cancer. *Clinical Cancer Research* 25(16), 5002 (2019).

* of interest – highly relevant paper covering TME and multi-omics

89. Mcgrail DJ, Federico L, Li Y *et al*. Multi-omics analysis reveals neoantigen-independent immune cell infiltration in copy-number driven cancers. *Nat Commun* 9(1), 1317-1317 (2018).
90. Kan Z, Ding Y, Kim J *et al*. Multi-omics profiling of younger Asian breast cancers reveals distinctive molecular signatures. *Nat Commun* 9(1), 1725-1725 (2018).
91. Buisseret L, Garaud S, De Wind A *et al*. Tumor-infiltrating lymphocyte composition, organization and PD-1/ PD-L1 expression are linked in breast cancer. *Oncoimmunology* 6(1), e1257452 (2017).
92. Barroso-Sousa R, Jain E, Cohen O *et al*. Prevalence and mutational determinants of high tumor mutation burden in breast cancer. *Annals of Oncology* 31(3), 387-394 (2020).

** of considerable interest – a very recent original research article on nearly 4000 breast cancer patients covering tumour mutational burden

93. Chen Z, Wen W, Bao J *et al*. Integrative genomic analyses of APOBEC-mutational signature, expression and germline deletion of APOBEC3 genes, and immunogenicity in multiple cancer types. *BMC Medical Genomics* 12(1), 131 (2019).
94. Burns MB, Lackey L, Carpenter MA *et al*. APOBEC3B is an enzymatic source of mutation in breast cancer. *Nature* 494(7437), 366-370 (2013).
95. Richters MM, Xia H, Campbell KM, Gillanders WE, Griffith OL, Griffith M. Best practices for bioinformatic characterization of neoantigens for clinical utility. *Genome Medicine* 11(1), 56 (2019).

* of interest – a paper detailing neoantigen prediction and characterisation from bioinformatics to the clinic

96. Rizvi NA, Hellmann MD, Snyder A *et al*. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 348(6230), 124-128 (2015).
97. Van Allen EM, Miao D, Schilling B *et al*. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 350(6257), 207-211 (2015).
98. Peng M, Mo Y, Wang Y *et al*. Neoantigen vaccine: an emerging tumor immunotherapy. *Molecular Cancer* 18(1), 128 (2019).
99. Liu S-H, Shen P-C, Chen C-Y *et al*. DriverDBv3: a multi-omics database for cancer driver gene research. *Nucleic Acids Research* 48(D1), D863-D870 (2019).
100. Xie B, Yuan Z, Yang Y, Sun Z, Zhou S, Fang X. MOBCdb: a comprehensive database integrating multi-omics data on breast cancer for precision medicine. *Breast Cancer Res Treat* 169(3), 625-632 (2018).
101. Schneider L, Kehl T, Thedinga K *et al*. ClinOmicsTrailbc: a visual analytics tool for breast cancer treatment stratification. *Bioinformatics* 35(24), 5171-5181 (2019).
102. Subramanian I, Verma S, Kumar S, Jere A, Anamika K. Multi-omics Data Integration, Interpretation, and Its Application. *Bioinform Biol Insights* 14 1177932219899051-1177932219899051 (2020).

** Of considerable interest – comprehensive paper covering different ways to analyse multi-omics data