Precision Clinical Medicine, 1(1), 2018, 29–48

doi: 10.1093/pcmedi/pby007
Advance Access Publication Date: 14 June 2018
Review

REVIEW

Characterization of cancer genomic heterogeneity by next-generation sequencing advances precision medicine in cancer treatment

Jialing Zhang[1,*,§], Stephan Stanislaw Späth[2,§], Sadie L. Marjani[3], Wengeng Zhang[4] and Xinghua Pan[5,6,1,*]

[1]Department of Genetics, Yale School of Medicine, Yale University, New Haven, CT USA, [2]University of Tübingen, Tübingen, Germany, [3]Department of Biology, Central Connecticut State University, New Britain, CT, USA, [4]Precision Medicine Key Laboratory of Sichuan Province & Precision Medicine Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China, [5]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Southern Medical University, and [6]Guangdong Provincial Key Laboratory of Single Cell Technology and Application, Guangzhou, Guangdong Province, China

*Correspondence: Jialing Zhang, Jialing.zhang@yale.edu; Xinghua Pan, Panvictor@smu.edu.cn

Abstract

Cancer is a heterogeneous disease with unique genomic and phenotypic features that differ between individual patients and even among individual tumor regions. In recent years, large-scale genomic studies and new next-generation sequencing technologies have uncovered more scientific details about tumor heterogeneity, with significant implications for the choice of specific molecular biomarkers and clinical decision making. Genomic heterogeneity significantly contributes to the generation of a diverse cell population during tumor development and progression, representing a determining factor for variation in tumor treatment response. It has been considered a prominent contributor to therapeutic failure, and increases the likelihood of resistance to future therapies in most common cancers. The understanding of molecular heterogeneity in cancer is a fundamental component of precision oncology, enabling the identification of genomic alteration of key genes and pathways that can be targeted therapeutically. Here, we review the emerging knowledge of tumor genomics and heterogeneity, as well as potential implications for precision medicine in cancer treatment and new therapeutic discoveries. An analysis and interpretation of the TCGA database was included.

Key words: Genomics; heterogeneity; next-generation sequencing; cancer treatment; precision medicine

[§]These authors contributed equally to this work.

Received: 23 March 2018; **Revised:** 10 May 2018; **Accepted:** 21 May 2018

© The Author(s) [2018]. Published by Oxford University Press on behalf of West China School of Medicine & West China Hospital of Sichuan University. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Tumors are marked by high levels of heterogeneity and out-of-control cell growth. They encompass an competing ecosystem, comprising a remarkable number of cancerous and unaffected cell sub-populations, including tumor-cell-related epithelial cells, infiltrated immune cells, fibroblasts, mesenchymal stroma/stem cells (MSCs), along with the surrounding endothelium of blood vessels (Fig. 1).[1] At the cellular level, gradual tumor development and progression appears to follow a classical evolution-like process following a step-wise accumulation of selected genomic or epigenetic alterations, which in turn lead to positive selection and expansion of certain cell lineages while other cell populations are depleted (Fig. 2 and Fig. 3).[2,3] This process, termed clonal evolution of tumors, or the stochastic model, is a dynamic process leading to continuous tumor remodeling with distinct dimensions of heterogeneity (Fig. 2b).[2,4,5] Conversely, the cancer stem cell model (CSC model or hierarchical model) is the other main mechanism that drives cancer progression, with either single or multiple progenitors (Fig. 2b). Interestingly, the heterogenetic patterns established with either the stochastic model or the CSC model are similar and hard to distinguish without identification, and in reality, they often co-occur. Indeed, CSCs usually are the cells that at a different lineage stage acquired mutations or epigenetic alternations that caused the cells to grow unchecked and form the tumor population at the clonal level.[6]

There are numerous recognized heterogeneity contributing and/or selection factors. For example, artificial

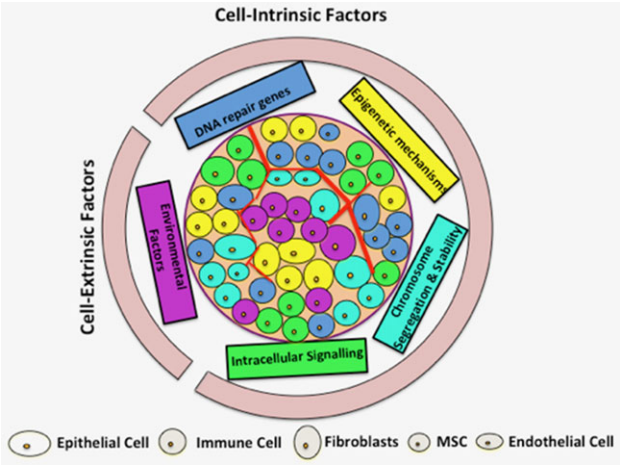

Figure 1. Interplay of key contributing factors to tumor heterogeneity. Both cell-intrinsic and cell-extrinsic factors contribute to tumor heterogeneity. Key cell-intrinsic factors include mutation, DNA-repair genes, epigenetic mechanisms, chromosome segregation and stability, as well as intracellular signaling. Non-genetic or phenotypic variations as a result of contributing cell-intrinsic factors are depicted by different cytoplasmic colors. Cell-extrinsic mechanisms affect and contribute to the unequal microenvironment, indirectly contributing to tumor heterogeneity. Multiple cell types and different inter- and intra-cell interactions within a tumor may exist (only representatives are shown here), hence selectively contributing to tumor heterogeneity.

intervention by chemotherapy or radiotherapy can positively act on the cancer evolution process by reshaping tumor cell populations at genomic, epigenomic, transcriptomic, and proteomic levels, ultimately leading to different phenotypic properties (Fig. 3).[7] Furthermore, cancer cell interaction with the surrounding microenvironment, given the complexity within and outside the cancer cell, is known to contribute to tumor heterogeneity.[1]

Based on numerous previous studies in multiple malignancies, tumor heterogeneity can be classified into inter-tumor (between tumors from different patients) or intra-tumor (within a single tumor, or tumor of a given patient) heterogeneity (Fig. 2a), based on specific molecular biomarker patterns (Table 1).[4,8] Inter- or intra-tumor heterogeneity marks a key challenge in oncology, with significant implications for selecting specific biomarkers and/or primary gene mutations to guide clinical decisions for precision cancer therapies.[2] The identification of alternatively expressed genes and multiple inter-/intra-cellular signaling pathways that drive phenotypic variation in multiple tumor types will also aid in the development of precise therapeutic approaches.

Genomic profiling technology, that is genome-wide next-generation sequencing (NGS), is increasingly used to uncover different aspects of genomic heterogeneity in many types of human diseases, including cancer.[9,10] The Cancer Genome Atlas (TCGA) project was a comprehensive and coordinated effort to accelerate our understanding of the molecular basis of multiple cancer types through application of genome-wide analysis technologies. The resulting data yielded insights into the close ties between tumor genetics and the evolutionary history of cellular processes across different cancer types.[11] In addition, the above-mentioned discoveries have significantly expanded our understanding of cancer at the molecular level. Evidence of this can be seen in the extensive application of NGS in cancer diagnosis and prognosis prediction in clinical settings, as well as a dramatic increase in the number of new drug discoveries that target specific biological pathways and/or genes that are studied in ongoing clinical trials. Furthermore, there has also been a significant acceleration in the use of NGS to create genomic signatures for use in precision medicine.[12,13]

Highly promising and constantly refined single-cell sequencing (SCS) technologies offer an ultimate solution for tackling the previously encountered limitations of intra-tumor heterogeneity analysis.[5] Simply put, SCS involves two major steps: (i) single- or multiple omics profiling of a large number of single cells, and (ii) classifying each tumor into different sub-populations from multiple spatial regions within a tumor biopsy with the use of sophisticated bioinformatics tools. These steps allow prediction of potential molecular relationships among these sub-populations within a single tumor biopsy. Combined with the serial spatial sampling set from a given tumor, SCS allows tracing of existing tumor cell lineages, and elucidation of potential therapeutic failure and resistance mechanisms, to further reveal the intricacies of tumor

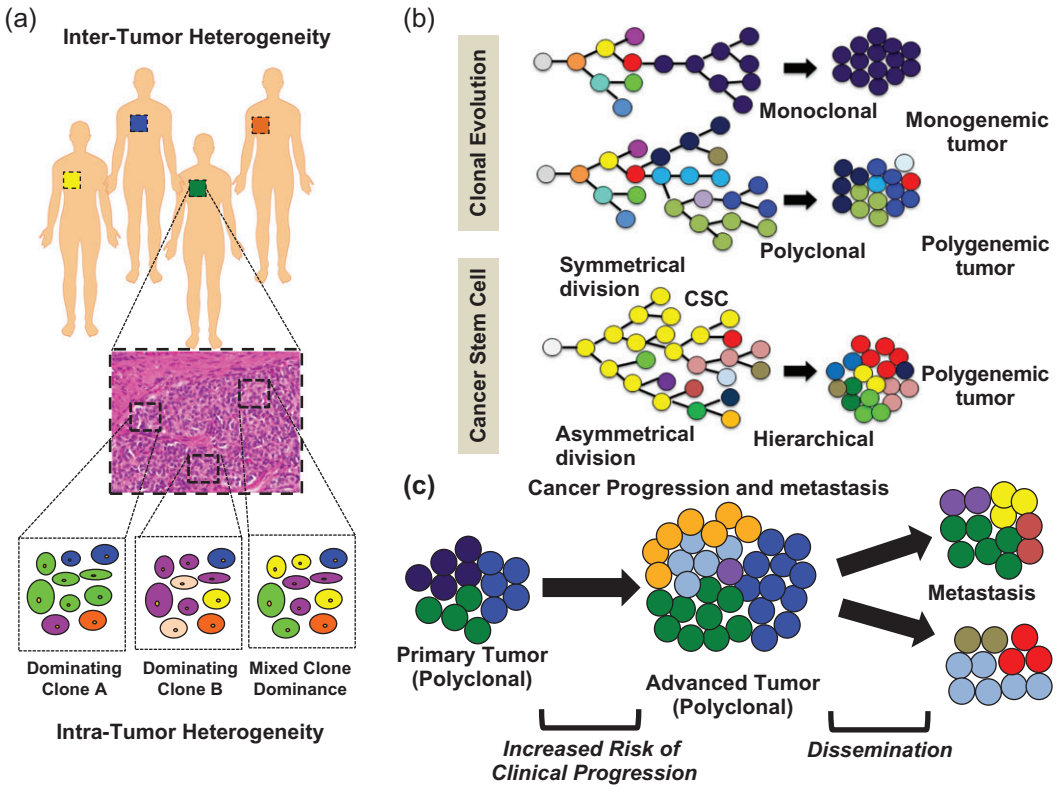

Figure 2. Contribution of tumor heterogeneity in cancer progression and metastasis. (a) Graphical representation of inter- and intra-tumor heterogeneity origins at macroscopic and microscopic levels. (b) Graphical summary of the two recognized heterogeneity models: clonal (stochastic) evolution and cancer stem cell (CSC), involving either monoclonal evolution or single progenitor, and polyclonal evolution or multiple progenitors, linking tumor cellular paths to different tumor heterogeneity. (c) Contributing role of tumor heterogeneity with respect to cancer progression and metastasis.

evolution.[14,15] The aim of this review is to discuss the contribution of heterogeneity to cancer development and treatment, and to examine the potential implications and limitations of NGS in deciphering tumor biology, along with its clinical translation in precision medicine.

Articles associated with large-scale genomic studies and TCGA, reviews, and related new clinical trials for most common types of cancer published between January 1, 2012 and December 31, 2017 were collected using PubMed and accessible public databases. The cBioPortal web resource tool (http://cbioportal.org) was used for cancer genomic data evaluation, including somatic mutations, DNA copy-number alterations, mRNA and microRNA expression, DNA methylation, and protein and phosphoprotein abundance. This tool allows users to query genetic alterations for each gene and sample, as well as hypothesis testing concerning recurrence and genomic gene alteration events in various common cancers. ClinicalTrials.gov is the largest clinical trial database, currently holding registrations from more than 195 countries around the world, allowing insights into current ongoing clinical trials. Key word searches included genomics, heterogeneity, clinical features, drug resistance, clinical trials, and phase I-III. Further inclusion criteria for published genomic studies in this manuscript included: (i) a sample size of more than 120 patients for genomic studies, and (ii) at least 15 patient participants in clinical trials.

Cancer genomic heterogeneity associated with clinical features

Since the discovery by evolutionary biologist Julian Huxley in 1958, there have been remarkable advances in the knowledge of genomic diversity and single tumor heterogeneity.[2] Today, there are many recognized factors contributing to genetic instability, including mutation of DNA repair genes directly or indirectly responsible for chromosomal stability, exposure to environmental mutagens, epigenetic mechanisms, as well as defects in chromosomal segregation, ultimately driving carcinogenesis (Fig. 1).[16,17] Another important issue to mention is that there are different types of genetic instability (i.e., deletion, amplification, point-mutations, etc.), which contribute to the high variability of cancer genomes, such as promoting genetic heterogeneity and ultimately differences in treatment response.[2,18,19]

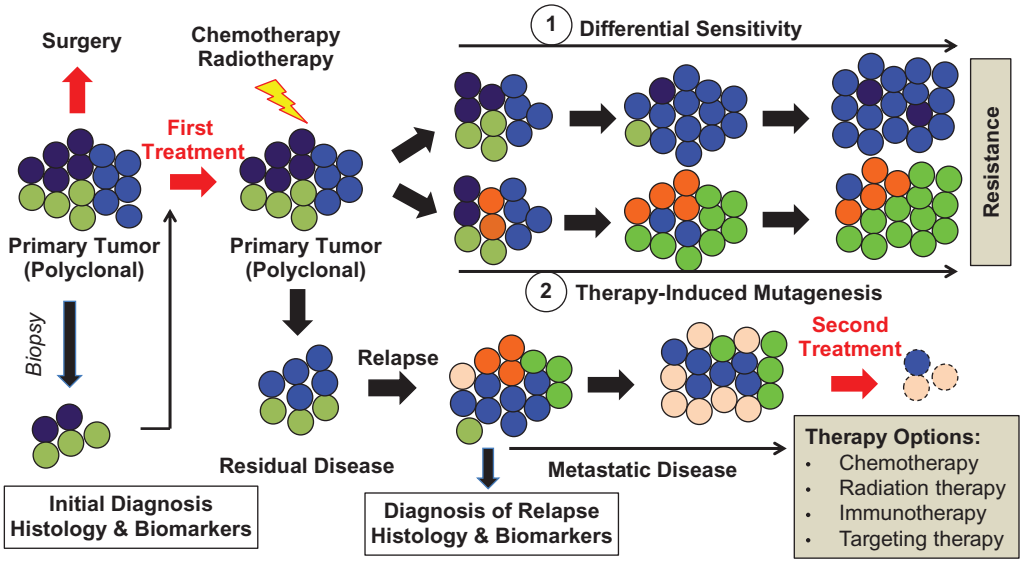

Figure 3. Role of tumor heterogeneity in biomarker prediction and tumor resistance to clinical therapy. Initial cancer diagnosis and first treatment depends on initial cell and molecular characterization, derived from a small tumor fraction (biopsy, here figure shows a complete representation, but in some cases, it may be biased). In most cases, the current first-line treatments can successfully eliminate dominating cancer clones, with the cost of selecting resistant tumor clones through either differential sensitivity (1) or therapy-induced mutagenesis (2). These resistant clones are capable of driving disease progression and eventually metastasis. Hence, the clonal composition of metastatic lesions may significantly differ from clones in the primary tumor. As a result, initial treatment choice may not be effective in progressive metastatic disease. This necessitates a new diagnosis and additional comparative steps after relapse, prior to second and usually combined treatment options (i.e., immunotherapy, selective pathway component targeting and/or gene therapy) (Adapted from Tellez-Gabriel *et al.*, 2016; doi:10.3390/ijms17122142).

Identification of genomic heterogeneity in pan-cancer studies

Extensive cancer genome studies have established a comprehensive landscape of genomic and epigenetic heterogeneity, with a strong link to initiation and progression in major cancers.[16] Recent data obtained from inter- and intra-tumor comparisons (Fig. 1a and b) link tumor heterogeneity to many types of malignant disease, that is lung,[20] breast,[21] prostate,[22] myeloma,[23] glioblastoma,[24] and colorectal cancers (CRCs),[25] as well as leukemia.[26] Molecular and phenotypic aberrant variations are not only common between tumors of different tissue and cell types, but also within a tumor derived from the same tissue or cell type within an individual patient.[12,16] For example, a study across 27 cancer types, including 3083 tumors and normal tissue pairs identified a total of 373 909 non-silent coding mutations by whole exome or whole genome sequencing.[27] A subsequent comparison revealed a 1000-fold difference between individual patient mutation rates within or across selected cancer types.[27] This evidence suggests that only a minority of these genes is essential for tumor development, with the majority having no significant biological impact (Table 1).[27] Intriguingly, several recent studies highlighted remarkably divergent patterns of genetic alterations in primary tumors when compared with metastases obtained from the same patient, where the metastatic tumors had additional mutations that were not present in the primary tumor (Table 1).[27–29] Essentially, the scientists hypothesized that all cells within a tumor have an equal potential to maintain and advance the tumor to metastasis, pending the acquisition of the necessary capability (Fig. 2c).[30] A TCGA study comprising 178 lung squamous cell carcinoma (LSCC) and normal pairs, identified a total of 360 exonic mutations, 165 genomic rearrangements, and 323 segments of copy alteration within a given tumor.[20] Statistical analysis uncovered 18 commonly mutated genes in 178 LSCCs, with *TP53* being among them (Table 1).[20] A very recent pan-cancer analysis, comprising over 3300 tumors, revealed a diverse genomic heterogeneity landscape across nine cancer types with a notable tendency for highly heterogeneous tumors to have lower levels of immune cell infiltration or T cell infiltration.[31] Cancers arise when a sufficient number of mutations have occurred in any given tumor cell pool.[32] These inevitably lead to accumulation of additional mutations within single cells that confer growth and survival advantages. Eventually, these cells will progressively give rise to new more aggressive progeny (Fig. 3).[33,34] Furthermore, multiple studies also revealed that a single mutation in one gene (i.e., *KRASG12D*, *BRAFV600E*) could induce a quick and sufficient malignant transformation in corresponding tissues of several tumor types (Table 1).[35,36] A significant association of high-level heterogeneity and poor survival was evident for lower grade glioma, prostate-, clear cell kidney carcinoma, head and neck-, as well as breast cancers, with borderline significance for melanoma.[18,31,37]

Table 1. Identification of significant genomically altered genes from published TCGA data in 12 common cancer types.

Cancer type	Sample size	Significantly altered genes	Reference
Glioblastoma	206	*TP53,ERBB2,NF1,PARK2,AKT3,FGFR2,IRS2,PTPRD,MLH1,MSH2,MSH6,PMS2,PIK3R1*	doi:10.1038/nature07385
Lung squamous cell carcinoma	178	*TP53,CDKN2A,PTEN,PIK3CA,MLL2,NOTCH1,RB1,HLA-A,NFE2L2,KEAP1*	doi:10.1038/nature11404
Lung adenocarcinoma	230	*TP53,NF1,RIT1,RBM10,ERBB2,MAP2K1,NRAS,HRAS,NKX2-1,TERT,MDM2,KRAS,EGFR,BRAF,PIK3CA,STK11,KEAP1, MET,CCNE1 CCND1, TERC,MECOM*	doi:10.1038/nature13385
Colon rectal cancer	276	*APC,TP53,KRAS,PIK3CA,FBXW7,SMAD4,TCF7L2,NRAS,CTNNB1 SMAD2,FAM123B,IGF2,NAV2,MYC,TGFBR2,BARF, MSH3,CASP8,CDC27,MAP7,PTEN,SOX9,ARID1A,FAM123B*	doi:10.1038/nature11252
Breast cancer	510	*PIK3CA,PTEN,AKT1,TP53,GATA3,CDH1,RB1,MLL3,MAP3K1,CDKN1B,TBX3,RUNX1,CBFB,AFF2,PIK3R1,PTPN22,PTPRD, NF1,SF3B1,CCND3*	doi:10.1038/nature11412
Ovarian carcinoma	489	*TP53,BRCA1,BRCA2,RB1,NF1,FAT3,CSMD3,GABRA6,CDK12,NOTCH,FOXM1,BRAF,PIK3CA,KRAS,NRAS,CCNE,MYC, ZMYND8,IRF2BP2,PAX8,TERT,ID4*	doi:10.1038/nature10166
Endometrial carcinoma	373	*PTEN,CTNNB1,PIK3CA,ARID1A,PPP2R1A,KRAS,MYC,ERBB2,CTNNB1,CCNE1,FGFR3,SOX17,TP53,PTEN,ARID5B,PIK3R1, FBXW7,POLE*	doi:10.1038/nature12113
Urothelial bladder carcinoma	131	*TP53,CDKN2A,FGFR3,PIK3CA,TSC1,RB1,HRAS,MLL2,CDKN1A,ERCC2,STAG2,RXRA,NFE2L2,ARID1A,KDM6A,EP300, FGFR3,PPARG E2F3, EGFR, CCND1,MDM2*	doi:10.1038/nature12965
Clear cell renal cell carcinoma	446	*VHL,PBRM1,BAP1,SETD2,HIF1A,PRKCI,MDS1,EVI1,MDM4,MYC,JAK2,CDKN2A,PTEN,NEGR1,QKI,CADM2,ARID1A, SMARCA4,PBAF*	doi:10.1038/nature12222
Gastric adenocarcinoma	295	*TP53,KRAS,ARID1A,PIK3CA,ERBB3,HLA-B,JAK2,PD-L1,PDCD1LG2,PTEN,SMAD4,CDKN2A,CDH1,RHOA*	doi:10.1038/nature13480
Head and neck cancer	279	*PIK3CA,TRAF3,E2F1,CDKN2A,HRAS,CASP8,NOTCH1,AJUBA,FAT1,NFE2L2,TP63,SOX2,EGFR,ERBB2,FGFR1*	doi:10.1038/nature14129
Cervical cancer	228	*APOBEC,SHKBP1,ERBB3,CASP8,HLA-A,TGFBR2,PD-L1,PDCD1LG2,BCAR4,KRAS,ARID1A,PTEN,PIK3CA,EP300,FBXW7, HLA-B,NFE2L2,MAPK1*	doi:10.1038/nature21386

Identification of genomic heterogeneity in hematological malignancy

The molecular pathogenesis of acute myeloid leukemia (AML) has been studied by applying cytogenetic analysis tools for more than three decades.[38] Characterization of AML genomes by next-generation sequencing has revealed that these tumors exhibit a relatively low recurrent somatic mutation rate, compared with most other cancers, with an average of only 13 identified mutations in AML associated genes (i.e., *DNMT3A*, *FLT3*, *NPM1*, *IDH1/2*, *RUNX1*, and *CEBPA*), along with recently discovered AML pathogenesis implicated genes (i.e., *U2AF1*, *EZH2*, *SMC1A*, and *SMC3*).[38–40] These mutations mainly enhance proliferation and survival of hematopoietic progenitors through activation of signaling pathways (Fig. 4).[39] A second class of mutated genes in AML includes transcription factors, such as *CEBPA* and *RUNX1*, which were found in ~20% of *de novo* normal cytogenetic AML, with short overall survival and relapse-free survival.[40] Mutations in *FLT3*, *NPM1*, and *CEBPA* have been shown to have a significant prognostic impact, which ultimately resulted in their inclusion within the risk stratification system of European Leukemia patients and their use in standard-of-care testing.[38,41] *TP53* mutation is frequently associated with therapy-related myeloid neoplasm and adverse prognostic impact.[40] Somatic mutations in the epigenetic modifiers, *DNMT3A*, *IDH1/2*, and *TET2*, are considered initiating AML mutations.[42] *NPM1* mutations confer a favorable prognosis only in the presence of a co-occurring *IDH1* or *IDH2* mutation. *IDH1* and *IDH2* inhibitors are currently being tested in clinical trials.[18]

Diffuse large B cell lymphoma (DLBCL) is the most common form of lymphoma in adults, accounting for 30-40% of newly diagnosed non-Hodgkin's lymphomas (NHL).[43] Historically, DLBCL has been divided into three molecular subtypes, including germinal center B cell-like (GBC), activated B cell-like (ABC), and the primary mediastinal B cell lymphoma (PMBL), with all exhibiting a striking heterogeneity in gene expression profiles as well as clinical outcomes.[44] Deep sequencing identified 322 genes that were recurrently mutated in DLBCLs, including *ARID1A*, *MEF2B*, *PIK3CD*, and *PIK3R1*, with additional genes involved in the *NF-kB* pathway (i.e., *TNFAIP3*) and *Wnt* pathway (i.e., *WIF1*).[45] The pathogenic driver status of *CARD11* alteration was reported by the discovery of gain of function germline mutations that drive constitutive *NF-kB* activation.[46] The GCB subtype was characterized by a more favorable outcome and a spectrum of genetic alterations, which include *PTEN* deletion and *EZH2* and *TP53* mutations.[43] The ABC subtype has a less favorable outcome, being associated with a distinct genetic background, and marked by translocations, *BCL2* amplification and *MYD88* mutation, which occur in approximately 30% of patients.[47] DLCBL patients with *MYD88* mutations are significantly older than patients without these mutations. PMBL displays an amplification of *JAK2* in 50% of cases and recurrent deletion of *SOCS1*, which is a suppressor of *JAK* signaling.[44] The relationship between therapy and genetic alteration is likely to contribute to convergent evolution, where mutation-conferring resistance will become highly prevalent in subsequently relapsed disease (Fig. 2c). As aforementioned, the intensive application of high-throughput genomic analysis has enabled rapid progress in our understanding of genetic heterogeneity in hematologic malignancies. Altogether, these examples suggest that the promise of precision medicine is finally coming to fruition in the desired treatment of blood malignancies.

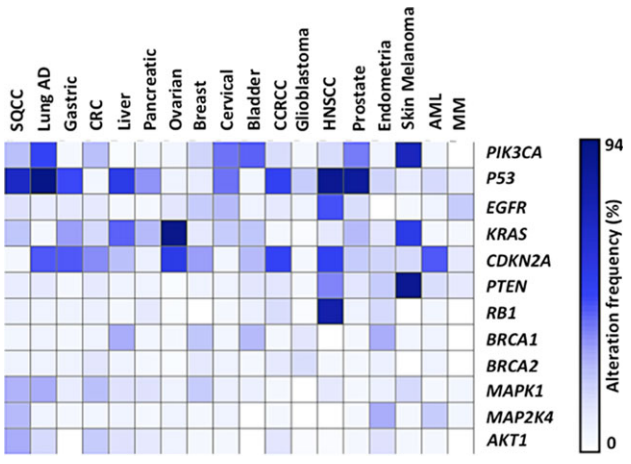

Figure 4. Recurrent somatic alterations across common tumor types. Heatmap of significant genes that were genetically altered across the 18 most common cancers, as evaluated by the TCGA project. Percentage of alteration frequency (white = low to blue = high) for the genes is shown.

Identification of genomic heterogeneity in solid tumors

Lung cancer is the leading cause of solid cancer-related mortality worldwide.[20] The discovery of recurrent mutations in *EGFR* kinase and *ALK* genes has led to a remarkable change in lung cancer treatment.[48] Targeting mutations in *BRAF*, *AKT1*, *ERBB2*, and *PIK3CA* has achieved great success in cancer therapy.[48] Recently, the comprehensive TCGA study of lung cancer from three large cohorts of patients comprising NSCLC, adenocarcinoma (AD), and squamous cell carcinoma (SQCC) characterized the presence of complex genomic alterations in these cancers.[49] Differential activity of PI3K/AKT/mTOR and MAPK pathways was present across NSCLS genomic subtypes.[49] The activation of p38/MAPK and mTOR pathways within a subset of lung AD, compared with other subtypes of lung AD and SQCC, was conducted. Significant somatic copy number alterations for the following genes, *MDM2*, *KRAS*, *EGFR*, *MET*, *CCNE*, were found in lung AD, with gene amplifications strongly dominating (Table 1 and Fig. 4). Interestingly, three AD associated subtypes expressed several immune

checkpoint genes, commonly associated with tumor cells or gene products known to interact with T cells (i.e., *PD1*, *PDL1*, *PDL2*, *CD3*, and *CTL4*), and have been nominated as potential therapeutic targets. Gender in lung AD is significantly correlated with gene mutation patterns (*RBM10*) (Table 1). The lung AD subtype appears to share similar gene patterns with many other cancer types, including CRC, stomach, pancreatic, breast, and liver cancers (Table 1). As expected, lung SQCC cancers also share many alterations (i.e., *PIK3CA*, *PTEN*, *TP53*, *CDKN2A*, and *RAS*) with head and neck, bladder, as well as cervical cancers (Fig. 4). A partial sharing with a multi-tissue squamous molecular subtype (Table 1 and Fig. 4) is also evident, marked by high expression of both *SOX1* and *TP63* genes, providing further evidence of common dysfunction in cell cycle control. TCGA further revealed that *PIK3CA* is amplified or mutated in ~34% of HPV negative and 56% HPV positive head and neck squamous cell carcinoma (HNSCC) tumors (Table 1 and Fig. 4), implicating the PI3K pathway in promoting growth factor dependent or independent growth, as well as the commonly observed EGFR therapy resistance.[50] It also promotes preferential expression of an oncogenic *ΔNP63* gene isoform of *TP63* encoded on chromosome 3 and involvement in squamous differentiation. Furthermore, a subset of ~22% of HPV positive HNSCC tumors had a notable 14q32.32 deletion or inactivating mutations in the *TNFR associated factor (TRAF3)* gene, with strong implications in suppressing survival of myeloid cancers and HPV positive HNSCC cell lines (Table 1).[50–52]

Approximately 15% of CRC display a high level of microsatellite instability (MSI), caused by germline mutations in one or more DNA mismatch repair (MMR) genes, as well as somatic inactivation of the same pathway.[53] Patients with early stage MSI CRC tumors have a better prognosis, compared with those harboring microsatellite stable (MSS) tumors. It is widely recognized that multiple genetic pathways (i.e., *Wnt*, *RAS-MAPK*, *PI3K*, *TGF*, *TP53*, and DNA mismatch-repair) are altered between benign and malignant lesions in CRC.[54] As expected, the TCGA project identified 24 significantly mutated genes, including *APC*, *TP53*, *SMAD4*, *SOX9*, and *FAM123B* (Table 1 and Fig. 4). Amplification of *ERBB2* and the newly discovered *IGF2* amplification was also observed, with promising drug-targetable potential. Mutated *APC*, *TP53*, *KRAS*, and *SMAD4* genes revealed a strong association with metastasis.[25] In early CRC stages, *SMAD4*, *TP53*, and *APC* appear to only display a very weak association with the disease outcome.[25] *APC* is a tumor suppressor gene and its mutation is known to regulate growth advantage in epithelial cells, ultimately leading to small adenoma formation.[54] Subsequently, *KRAS* and *BRAF* mutations provide a second round of favorable cell expansion, resulting in large adenoma transformation.[25,55] Eventually, mutations in *PIK3CA*, *SMAD4*, and *TP53* genes generate a malignant tumor, with a high potential for invasion and metastasis.[25,54]

Genomic analysis of the main breast cancer subtypes revealed that its cause was also associated with different subsets of molecular heterogeneity (Table 2).[56] Clinically, this heterogeneity of breast cancer can be broadly categorized into three basic therapeutic groups: (i) the *estrogen receptor* (*ER*) positive group is the most numerous and diverse, with several genomic tests (*Ki-67*) to assist in predicting the outcome for *ER* positive patients receiving endocrine therapy[57]; (ii) triple-negative breast cancer (*ER-, progesterone receptor* (*PR*)- *and human epidermal growth factor receptor-2* (*HER2*)) is an optimal patient group for chemotherapy options only, marked by increased incidence of germline *BRCA1* mutations[58]; and (iii) basal-like breast cancer typically lacks expression of the molecular targets that confer responsiveness to highly effective targeted therapies, such as Tamoxifen and Aromatase inhibitors or Trastuzumab. The TCGA project revealed that somatic mutations in only three genes (i.e., *TP53*, *PIK3CA*, and *GATA3*) occurred at >10% incidence across all breast cancer types (Table 2).[56] Deletion or translocation events in tumor suppressor genes, such as *AKT3* and *MAG13*, lead to functional abnormalities and initiate breast tumorigenesis.[56] High levels of *APOBEC3B* gene expression have been shown to be associated with disease-free survival and overall survival outcomes in patients with *ER+* breast cancer.[59] Recent studies on breast cancer uncovered a list of driver genes, such as *CCND1*, *RB1*, *ERBB1*, *FGFR1*, *MYC*, and *PTEN* (Table 1 and Fig. 4).[56] Variable frequencies of *HER2* gene amplification between primary tumors and their metastatic tumor or circulating tumor cells in advanced breast cancer have also been reported.[60,61] These studies suggest that a subset of patients with initial *HER2* negative primary tumors may develop *HER2* positive circulating tumor cells during disease progression, although the exact mechanism is still to be elucidated. Several studies also revealed a marked association between prior history of breast carcinoma and secondary acquired mutations in either primary or recurrent ovarian carcinomas, with breast carcinoma often preceding the ovarian carcinoma by many years.[62,63] Therefore, increased focus on driver mutations in tumorigenesis would provide critical insights for personalized therapeutics in cancer treatment. The identification of significant genomic heterogeneity

Table 2. Genomic heterogeneity in sub-types of breast-like cancer from the TCGA project.

Mutated genes	Luminal A (%)	Luminal B (%)	HER2(+) (%)	Basal-like cancers (%)
PIK3CA	45	29	39	9
TP53	12	29	72	80
MAPK1	13	5	4	0
MAP2K4	7	2	2	0
AKT1	4	2	2	0
PTEN	4	4	2	1
RB1	0.40	3	0	4

from published TCGA project data derived from 12 cancer types is summarized in Table 1.

Association of cancer genetic heterogeneity and therapeutic failures

Genetic heterogeneity is a prominent contributor to therapeutic failure, with increased likelihood of resistance to future therapies. This generates a diverse cell population during tumor development and progression, representing a key determining factor for variation in tumor therapeutic response (Fig. 3).[8,16] Resistance to single drug targeting therapies is frequent in cancer, and near universal in major metastatic carcinomas. *KRAS* mutations are known to confer resistance to *EGFR* targeting in cancer treatment.[64] The well-documented mechanisms of drug resistance to certain therapies are associated with alterations in signal transduction cascades, predominantly through activation of alternative or complementary pathways, often through molecular feedback loops.[65] Insights into the genomic landscape of some cancers, such as NSCLCs and breast cancers, have fueled a shift in the treatment paradigm towards the use of precise treatments.[49,66,67] Lung cancer patients with heterogeneous *EGFR* mutations appear to benefit less from the *EGFR* inhibitor Gefitinib than patients with homogeneous *EGFR* mutations.[68] Mutated *EGFR* was shown to be mediated by selected cells that harbor the *EGFR* gatekeeper mutation (T790M) and/or *MET* gene amplification.[69,70] The oncogenic *BRAF* amplification or *MEK1* mutation associated with resistance to *BRAF*-specific inhibitors in melanomas is also well documented.[71,72] However, $BRAF^{V600E}$ mutations are among the most commonly reported molecular alterations in melanomas, and *BRAF* is currently a promising therapeutic target.[71] Successful clinical trials of selective BRAF inhibitors (i.e., 'Vemurafenib') in $BRAF^{V600E}$ mutated versus non-mutated patient melanoma tumors support their substantial potential and clinical significance, together with patient-derived tumor genotyping, prior to appropriate treatment selection.[73] Chronic myeloid leukemia (CML) is a stem cell-like disease, marked by the presence of rare cell clones in about half of patients with unique *BCR-ABL* resistant mutations, possibly acquired after Imatinib treatment.[74] Furthermore, impairment of apoptotic cell death plays a major role in therapy resistance and relapse in acute lymphoblastic leukemia (ALL).[75] Recent studies have shown that apoptosis *protein inhibitor interacting protein kinase 1 (RIP1)* inhibitor 'Birinapant' potently induced cell death in patient-derived ALL cells both *in vitro* and *in vivo*.[76,77] Patients in leukemic relapse are notoriously difficult to treat because drug resistance of leukemic clones is an insurmountable obstacle to effective chemotherapy in AML.[78] Loss of tumor suppressor *BRCA1/2* gene heterozygosity highly sensitizes patients to DNA cross-linking agents (platinum drugs) in ovarian or breast cancer.[79] Predictive capability of platinum resistance through the presence of secondary *BRCA1/2* mutations in ovarian cancer has been documented in *in vitro* and *in vivo* studies.[63] CRC resistance to targeted therapy noted during disease progression is often occurring within 3-12 months after *EGRF* antagonist administration, demanding a change in the treatment choice.[80] Proposed mechanisms associated with the failure of improved outcomes in CRC patients were linked to microscopic residual disease and the absence of tumor neoangiogenesis after 'Bevacizumab' (anti-*EGFR* antibody) application, as well as the epithelial to mesenchyme transition phenotype after 'Cetuximab' (anti-EGFR antibody) treatment.[81] Furthermore, the expression of the *excision repair cross-complementation group 1 (ERCC1)* gene is under increased investigation as a potential resistance predictive marker to platinum compounds in CRC.[82] The benefit of chemotherapy is further increased with combined targeting therapies (i.e., 'Bevacizumab,' 'Cetuximab,' or 'Panitumumab') in CRC patients with *RAS*-wild type harboring tumors.[83] In addition, preliminary clinical data have revealed that *HER2* is amplified in around 5% of patients with *KRAS*-wild type metastatic CRC, suggesting that this patient subset may benefit from dual *HER2* inhibitors (i.e., 'Trastuzuman' and 'Lapatinib').[84]

Drug resistance mechanisms of selected tumor clones and the extremely intra-heterogeneous nature of the tumor are also widely accepted (Fig. 3).[85] This leads to significant practical difficulties in identifying the most aggressive or drug resistant clones to deliver targeted therapy, through well-established conventional bulk sequencing approaches.[11] At present, targeted therapeutic reagents are dependent upon biomarkers that are derived from primary tumor biopsies that are subjected to genomic sequencing. However, dramatic responses to initial therapy and relapse typically take place within 1-2 years following treatment initiation, which commonly arise from selective pressures created by the dynamic nature of the targeting agent.[86] Furthermore, drug response failure of sub-clones within certain cancer tissues substantially limits the ability to predict treatment response.[87] Our current understanding of heterogeneity extent in cancers is largely derived from bulk tumor specimen analysis. It should be noted that most bulk tumor samples are a mixture of non-malignant cells and diverse cancer cell sub-populations (Fig. 2 and Fig. 3). The implementation of single-cell analysis (SCA) technologies to study cancer heterogeneity has shown a strong potential to reveal genome-wide molecular profiles, regulation, and mechanisms, with unprecedented resolution.[5,88] This state-of-the-art methodology allows, with reliable precision, the isolation and characterization of individual cells among a heterogeneous cell mixture. It further grants an opportunity for future breakthroughs in understanding the dynamic genetic heterogeneity in tumors, along with cancer origin, progression, and clinical management.

Advances of SCA technology to uncover dynamic genetic heterogeneity

Over the past few years, technological advances at the single-cell level have made high-throughput sequencing of tumor genomes possible. An increasing number of reported single-cell studies demonstrate considerable cell-to-cell variability in apparently homogeneous populations. Application of SCA has greatly enhanced the power of systematic cancer heterogeneity characterization, resulting in significant mechanistic insights into tumor progression.

Single-cell gene expression analysis dates back to the early 1990s[89]; however, it is in the last decade that a significant advance has been achieved in SCA technology development (refer to reviews[14,90]). To date, it is becoming possible to assay a substantial number (>100) of secreted proteins, cell surface markers, signaling pathway components, and even metabolites at the single-cell level.[90] The most significant progress of SCA tool development is evident at the genomic, transcriptomic, epigenetic, and proteomic levels.[5,90] SCA tools significantly contributed to the identification and characterization of cancer stem cells. The success of scRNA-seq in this area was marked by the discovery of 'stemness'-like cells by analyzing transcriptome- and gene expression signatures of *in vivo* differentiated glioblastoma cells,[91] as well as metastatic breast tumors.[92] These observations support the theory that initial tumor stem cells may initiate and propagate metastatic cancer behavior.

As an application with a great clinical potential, SCA was applied to circulating tumor cells (CTCs). The previously impossible detection and characterization of CTCs, originating from tumors and at 1:10^9 ratio in the bloodstream, has been made possible through SCA techniques.[92] The previously unreliable method of using magnetic beads coupled to a cell surface ECAM (Epithelial Cell Adhesion Molecule) recognizing antibody, has been optimized for single-cell CTC isolation from whole blood, by considering factors such as microscopic imaging, cell size, and passive capture.[93,94] The elucidation of cancer progression through the comparison of genomic and transcriptomic derived CTC profiles has also been reported.[95,96]

By using single-cell adapted whole-genome sequencing, the discovery of the metastatic pathway (potentially because of differential CNV patterns) from lung cancer-derived CTCs was achieved.[97,96] The promising applications of non-invasive SCAs to study cancer development, as well as cancer therapy resistance following chemotherapy are also evident.[98] Currently, two prevalent therapy resistance theories exist: (i) adaptive resistance, where low frequency mutations in the original population are selected for and eventually rise in frequency during chemotherapy; or (ii) acquired resistance, where resistance-conferring mutations are directly linked to chemotherapy.[90] As a consequence, the main goal and use of SCAs in clinical settings has been detection and evaluation of mutational differences over time in CTCs (i.e., before and after treatment). Eventually, this will lead to insights into the mechanisms of therapeutic resistance development in various cancer types, with subsequent validation of the aforementioned resistance theories. The use of SCA technology to study the response of mutated $BRAF^{V600E}$ melanoma to RAF- or RAF/MEK combined inhibitors *in vitro* or *in vivo* led to the discovery of an overexpressed, and well-known *AXL* resistance marker, which was also linked to the adaptive resistance mechanism.[90,99] In another study, CTC tracking and subsequent whole-genome sequencing of prostate-derived cells, before or after androgen (AR)-targeted therapy, led to the discovery of two distinct resistant and AR amplified cell populations.[100] One of the populations was shown to be closely related to the cells prior to initiated therapy, supporting the adaptive resistance theory.[100] Furthermore, identification of heterogeneous resistance-conferring changes in the AR-independent Wnt signaling pathway could be derived using the scRNA-seq technique.[101] In addition, following 'Trastuzumab' treatment of *HER2* mutated breast tumor samples and subsequent STAR-FISH SCA, a close link between increased *PIK3CA* mutations and increased dispersion, as well as decreased frequency of *HER2* amplification and chemotherapeutic resistance, was detected.[102] The authors concluded that 'Trastuzumab' treatment has no benefit to patients who had already received chemotherapy and that the STAR-FISH approach could be used to predict poor prognosis.[102]

Clinical studies elucidating potential therapeutic significance of genetic heterogeneity for precision medicine

The subsequently mentioned studies further clarify the concept of tumor heterogeneity and its related pathogenesis, presenting a major area for new therapeutic approaches. Major subsets of molecular alterations in key pathways and driver mutations have interesting potential for targeting PI3K, mTOR, ERK/MAPK pathways, as well as checkpoint immunotherapy.

PI3K-AKT-mTOR inhibitors

The PI3K-AKT-mTOR pathway plays a critical role for many cellular functions, such as growth control, survival, and metabolism, and is known to be highly activated in human cancers.[103] Previous studies have shown that PI3K-AKT-mTOR pathway over-activation is associated with mutations and amplification of genes encoding receptor tyrosine kinases (i.e., *HER2* or *EGFR*), *PIK3CA* mutations, *PTEN* loss and/or mutation, and *KRAS* mutations during carcinogenesis (Table 1 and Fig. 4). A significant amount of effort has been put into development of drugs targeting several kinases throughout the phosphatidylinositol-3-kinase (PI3K) pathway for cancer therapy. Novel PI3K target inhibitors are currently being investigated in phase II and phase III clinical trials for various cancers (Table 3). There are different isoforms of PI3Ks, such as PI3K-α, β, γ,

Table 3. Summary of small molecule inhibitor clinical trials in human cancers. Data taken from http://clinicaltrials.gov/.

Drug	Combination	Sponsor	Tumor type	Sample size	Status	Recruitment Status	Clinical trial ID
PI3K inhibitors							
BAY80-6946 (Copanlisib)	-	Bayer	Lymphoma, Non-Hodgkin's	227	Phase II	Active	NCT01660451
BKM120	-	Hospices Civils de Lyon	Thyroid Cancers	47	Phase II	Active	NCT01830504
BKM120	-	SOLTI Breast Cancer Research Group	Triple Negative Metastatic Breast Cancer	50	Phase II	Completed	NCT01629615
BKM120		Centre Leon Berard	Metastatic Head and Neck Cancer Recurrent or Progressive	70	Phase II	Recruiting	NCT01737450
BKM120	Cetuximab	University of Chicago	Recurrent or Metastatic Head and Neck Cancer	30	Phase II	Active	NCT01816984
PQR309	-	PIQUR Therapeutics AG	Lymphoma, Malignant	72	Phase II	Recruiting	NCT02249429
	-		Endometrial Clear Cell Adenocarcinoma				
	-		Endometrial Adenosquamous Carcinoma				
BKM120	Trastuzumab	Novartis Pharmaceuticals	HER2-positive Primary Breast Cancer	50	Phase I/II	Completed	NCT01816594
BYL719	Paclitaxel	Priyanka Sharma	HER-2 Negative Breast Cancer	44	Phase I/II	Active	NCT02379247
Taselisib	Enzalutamide	Vanderbilt-Ingram Cancer Center	AR Positive Triple-Negative Metastatic Breast Cancer	73	Phase I/II	Recruiting	NCT02457910
Idelalisib	Entospletinib		Hematologic Malignancies	66	Phase I/II	Completed	NCT01796470
GSK2636771	Pembrolizumab	M.D. Anderson Cancer Center	Metastatic Melanoma and PTEN Loss	41	Phase I/II	Recruiting	NCT03131908
Everolimus	Exemestane	Novartis Pharmaceuticals	Metastatic Breast Cancer with ER+		Phase III	Completed	NCT00863655
Akt inhibitors							
Akt Inhibitor MK2206	-	National Cancer Institute (NCI)	Endometrial Adenocarcinoma	37	Phase II	Completed	NCT01307631
Akt Inhibitor MK2206	-	National Cancer Institute (NCI)	CRC	18	Phase II	Completed	NCT01802320
MK-2206 + AZD6244	-	National Cancer Institute (NCI)	Colorectal Neoplasms	21	Phase II	Completed	NCT01333475
mTOR inhibitors							
Everolimus	Vinorebine	AIO-Studien-gGmbH	Advanced Breast Cancer	139	Phase II	Completed	NCT01520103
BEZ235	-	Novartis Pharmaceuticals	Pancreatic Neuroendocrine Tumors (pNET)	31	Phase II	Completed	NCT01658436
Rapamycin	-	The University of Texas Health Science Center at San Antonio	Cancer of Breast	60	Phase II	Recruiting	NCT02642094
Everolimus	-	M.D. Anderson Cancer Center	Endometrial Cancer	270	Phase II	Recruiting	NCT02397083
Everolimus	-	University of Texas Southwestern Medical Center	Children With Recurrent or Progressive Ependymoma	18	Phase II	Recruiting	NCT02155920
Everolimus	-	National Cancer Institute (NCI)	Kidney Cancer or Renal Cancer	18	Phase II	Recruiting	NCT02504892
TAK-228	-	Fox Chase Cancer Center	Soft Tissue Sarcomas	33	Phase II	Recruiting	NCT02987959

Continued

Table 3. Continued

Drug	Combination	Sponsor	Tumor type	Sample size	Status	Recruitment Status	Clinical trial ID
AZD2014	-	Canadian Cancer Trials Group	Glioblastoma Multiforme	52	Phase II	Recruiting	NCT02619864
Everolimus	Cisplatin	Jenny C. Chang, MD	Triple Negative Breast Cancer	32	Phase I/II	Recruiting	NCT01931163
Everolimus	Sorafenib Tosylate	Alliance for Clinical Trials in Oncology	Thyroid Cancer	34	Phase I/II	Recruiting	NCT02143726
Everolimus	LEE011	Memorial Sloan Kettering Cancer Center	Neuroendocrine Tumors	41	Phase I/II	Recruiting	NCT03070301
Sirolimus+	Cisplatin	University of Washington	Bladder Cancer	21	Phase I/II	Completed	NCT01938573
Enzalutamide	LY3023414	Eli Lilly and Company	Prostate Cancer	144	Phase I/II	Recruiting	NCT02407054
ERK1/2 and MAPK inhibitors							
Regorafenib	-	Gerald Batist	Metastatic Colorectal Cancer	52	Phase II	Recruiting	NCT01949194
Vandetanib	-	Ronald Weigel	Invasive Breast Cancer	100	Phase II	Recruiting	NCT01934335
BVD-523	-	BioMed Valley Discoveries, Inc	Myelodysplastic Syndrome	53	Phase II	Completed	NCT02296242
TDM1	Abraxane, Lapatinib	Jenny C. Chang, MD	Metastatic HER2 Positive Breast Cancer	45	Phase I/II	Recruiting	NCT02073916
LY2228820	Radiotherapy + TMZ	Centre Jean Perrin	Newly Diagnosed Glioblastoma	50	Phase I/II	Recruiting	NCT02364206
Dabrafenib	Pazopanib hydrochloride	Manisha Shah	Unspecified Adult Solid Tumor	56	Phase 1	Active, not recruiting	NCT01713972
GSK2118436	GSK1120212	Novartis Pharmaceuticals	Cancer	430	Phase 2	Active, not recruiting	NCT01072175
NFκB inhibitors							
Pentoxifylline	-	Ramón Óscar González-Ramella, Ph.D	Pediatric Acute Lymphoblastic Leukemia	44	Phase II	Recruiting	NCT02451774
Dexamethasone	-	Emory University	Plasma Cell Myeloma	90	Phase II	Recruiting	NCT02765854
Ibrutinib	-	Icahn School of Medicine at Mount Sinai	Multiple Myeloma Patients	36	Phase II	Recruiting	NCT02943473
Lansoprazole	-	National Health Research Institutes, Taiwan	Early-stage HP(+) Gastric Pure DLBCL	30	Phase II	Recruiting	NCT02388581
Ibrutinib	Rituximab	Samsung Medical Center	EB+ Diffuse Large B-cell Lymphoma	24	Phase I/II	Recruiting	NCT02670616

and δ, with studied inhibitors known to inhibit one or more isoforms.[104] 'Idelalisib' (GS-1101 or CAL-101), a selective PI3K-δ inhibitor was approved in 2014 by the US FDA for treatment of various hematological malignancies, including chronic lymphocytic leukemia (CLL), relapsed B-cell non-Hodgkin's lymphoma, and relapsed small lymphocytic lymphoma.[105] 'Copanlisib' (BAY 80-6946), a PI3K inhibitor predominantly targeting PI3K-α and PI3K-γ isoforms was the second FDA-approved drug in 2017 to treat adult patients with relapsed lymphoma.[106] The BMK120 and BYL719 compounds are pan-PI3K inhibitors that have demonstrated preliminary selective activity in preclinical models of solid tumors.[104] Both compounds have shown favorable tolerability profiles with consistent on-target inhibition of PI3K. They have been studied as therapeutic targets either alone or in combination in phase II trials against solid tumors and hematologic malignancies (Table 3). PI3K-AKT-mTOR is the most frequently activated signaling pathway in breast cancer.[56] 'Everolimus' (RAD-001), a selective inhibitor against mammalian target of rapamycin (mTOR), has been investigated in a phase III clinical trial and in combination with 'Exemestane' trials (funded by Novartis, NCT00863655) for ER positive advanced breast cancer.[66] The results have shown that the median progression-free survival was 6.9 months with 'everolimus' plus 'Exemestane' and 2.8 months with placebo plus 'Exemestane'.[66] 'Buparlisib' (BKM120) is an oral pyrimidine-derived reversible pan-PI3K inhibitor with specific and potent activity against mutant PI3K-α, as well as wild-type PI3K-α, β, γ, and δ isoforms, but no inhibitory activity against the class III PI3K or mammalian target of Rapamycin (mTOR). A phase IB/II study has investigated combined 'Buparlisib' and 'Trastuzumab' (NCT01132664) treatment in relapsed HER2 (+) breast cancer that previously failed with 'Trastuzumab' alone (Table 3).[107,108] The data revealed that 'Buparlisib' and 'Trastuzumab' were well tolerated, with preliminary signs of clinical activity being observed in two partial responders and seven patients with stable disease. This promising outcome has led to further ongoing investigations of PI3K inhibitors in patients with HER2 (+), HER2 (-), and/or AR (+) triple negative metastatic breast cancer (NCT01816594, NCT02379247, NCT02457910) (refer to Table 3). PI3K-AKT-mTOR pathway alterations associated with *PIK3CA* mutation are evident in almost a third of HNSCC (Table 1 and Fig. 4). A number of first generation PI3K and mTOR inhibitors (i.e., 'Rapamycin', 'Temsorlimus' (CCI-779), 'Everolimus' (RAD-001)) have shown activity in *in vivo* preclinical models.[109] 'Alpelisib' (BYL719), specifically inhibits PIK3 in the PI3K/AKT kinase signaling pathway. 'Alpelisib' in combination with 'Cetuximab' have demonstrated synergistic activity in HNSCC cell lines, resulting in induced tumor regression in *PIK3CA* mutant HNSCC xenograft model.[109] Currently, two ongoing phase II clinical trials are assessing 'Buparlisib' (BKM120) in recurrent or metastatic HNSCC on 'Cisplatin'- and 'Cetuximab'-based chemotherapy in *PIK3CA*-mutated and wild-type patient cohorts (NCT01737450, NCT01816984, refer to Table 3). Loss of *PTEN* is associated with increased PI3K-AKT pathway activation and is commonly observed in up to 30% of melanomas, and frequently also observed in tumors with a concurrent activating BRAF mutation (Table 1 and Fig. 4). A phase I/II clinical trial of GSK2636771 in combination with 'pembrolizumab' is currently ongoing in patients with refractory (non-responsive to treatment) metastatic melanoma (NCT03131908, refer to Table 3). A summary of previous and ongoing clinical trials of individual, as well as combined PI3K, mTOR and AKT inhibitors (as registered in ClinicalTrials.gov) is provided in Table 3.

MARK/MEK inhibitors

The mitogen-activated protein kinase/extracellular signal regulated kinase (MAPK/ERK) signaling cascade is tightly regulated by phosphatases and bi-directional communication with other pathways, such as the AKT/mTOR pathway.[110] This pathway is vital for human cancer cell survival, dissemination, and drug resistance development. It is known to be frequently activated by a wide variety of receptors, including upstream genomic events and/or activation of multiple signaling events in solid and hematological malignancies.[103] Genomic tumor profiling has identified amplifications of several growth factor receptor genes, including *EGFR*, *ERBB2*, *IGF1R*, *FGFR1*, and mutations in *RAS*, *BRAF* and *MAPK/ERK* pathway genes that are ready for targeting in cancer treatment (Table 1, Table 3, and Fig. 4). Currently approved B-RAF kinase inhibitors (BRAFi) for melanoma treatment are being investigated either alone or in combination with other agents in many other tumor types (refer to Table 3). The clinical trial using CDK inhibitor 'LEE011' in combination with phase II BRAF inhibitor 'Encorafenib' (LGX818), with the aim to target key enzymes in the MAPK signaling pathway of *BRAF* mutant melanoma patients, was abandoned because of safety concerns (NCT01777776, refer to Table 3).[111] However, therapies targeting MAPK/ERK components appear to have variable responses, when used in different solid tumors, including breast cancer, CRC, and glioblastoma (Table 3). BRAF and MEK inhibition results in increased melanoma antigen expression, as observed in melanoma cell lines.[110] This phenomenon may increase tumor recognition by T-cells, with a strong potential to develop into a successful immunotherapeutic approach, warranting further exploration into combined approaches of immunotherapies and MAPK/ERK inhibitors.[112] Currently ongoing Phase II clinical trials using agents targeting BRAF and MEK kinases are summarized in Table 3.

Nuclear factor kappa-B (NF-κB) inhibitors

The implication of the NF-κB signaling pathway has been well established in recent decades in both physiologic

and pathologic conditions, including cancer.[113] The role of NF-κB in human cancer initiation, metastasis, and resistance to treatment has been exclusively investigated and has drawn particular attention. A significant number of human cancer genomic studies have revealed that NK-κB activation is highly associated with an inflammatory microenvironment and various oncogenic mutations.[114] It appears to be a key mediator in the crosstalk between inflammation and carcinogenesis. The NK-κB family consists of five master transcription factors, including NF-κB1, NF-κB2, RelA, RelB, and c-Rel, which bind to DNA and regulate gene transcription. The role of NF-κB in cancer development started to be closely investigated when several NF-kB family genes were found to harbor rare mutations in certain types of cancers, especially in hematopoietic malignancies.[114] As such, a large cohort study in DLBCL was characterized by preferential activation of the NF-κB pathway and subsequent nuclear expression of p50/p65 and p50/c-Rel dimers, compared with germinal B cell lymphocytes.[115] Amplifications and rearrangements in *c-Rel* genes are often detected in various non-Hodgkin's B cell lymphomas.[116] NF-κB also plays a significant role in metastasis of several solid tumors, including breast cancer, HNSCC, and lung cancer.[113] It has been shown that inhibition of NF-κB abolishes VEGF production and subsequent angiogenesis in a variety of conditions.[117] In addition, NF-κB induces the expression of anti-apoptotic genes, such as caspase-8 inhibitor FLIP, inhibitor of apoptosis genes *c-IAP1/2* and *XIAP*, as well as apoptosis regulating genes belonging to the Bcl-2 family. Furthermore, many oncogenic mutations in *EGFR*, *Ras*, *PI3K*, and *TP53* genes are known to contribute to NF-κB activation in cells derived from pancreatic, colorectal, and lung tumors, further warranting NF-kB targeting as a cancer therapy.[117,118] Recently, therapeutic agents specifically targeting the NF-kB pathway have been considered to be front-line therapy. 'Pentoxifylline' specifically targets c-Rel nuclear translocation and also inhibits NFAT, which is currently under investigation in a phase II clinical trial involving pediatric ALL patients (NCT02451774, refer to Table 2). 'Ibrutinib' selectively inhibits BCR and NF-kB singling, hence reducing cell proliferation in CLL patients that is characterized by prominent activation of NF-kB and BCR.[43,74] The phase I/II clinical trial applying 'Ibrutinib' either alone or in combination with EGFR inhibitor 'Rituximab' is being currently tested in DLBCL patients (NCT02670616, NCT02388581, refer to Table 3). Hundreds of natural and synthetic compounds have been reported to selectively inhibit NF-kB; however, their clinical application has shown little efficacy, except for certain types of lymphoma and leukemia.[119] There is evidence that various NF-kB inhibitors prolonged survival in NSCLC mouse tumor models, induced by *KRAS* and *TP53* compound mutations; however, resistant tumors appeared within several weeks.[120] Mechanisms that led to this resistance remain unclear. Nevertheless, NF-κB inhibitors still appear attractive, although combinations with other chemotherapies are currently considered a better choice.[121] Furthermore, NF-kB activation has been linked to sensitization to chemo- or radiation therapies, with a strong potential to serve as a biomarker.[121] Thus, clinical trials are currently investigating NF-kB activation as a biomarker in response to radio- and chemotherapies in patients with rectal- (NCT00280761) and gastric carcinomas (NCT01905969).

Immune checkpoint inhibitors

Recent exciting advancements in cancer treatment have been achieved in the field of immunotherapy. Vital fundamental discoveries over the last few decades have shown that the immune cells play a critical role in maintaining an equilibrium between immune recognition and tumor development, with the dual capacity of promoting and suppressing tumor growth.[122] It is well accepted that tumor cells derive from genetic instability, uncontrolled cell division, and reduced immunogenicity that allows tumors to evade the immune system.[123] These processes enable tumor cells to impair the immune system's capacity to eradicate them by immune suppressive effects or by loss of targetable antigen expression. Therefore, cancer immunotherapy involves use of naturally derived or synthetically generated components with the goal of activating the immune system to target the cancer.[123] Immune checkpoint inhibitors have demonstrated a considerably important breakthrough in the recent approval to treat solid tumor and hematologic malignancies in cancer immunotherapy.[124] The main concept of immune checkpoint targeting is to prevent receptors on the T cells and cancer cell ligands from binding to each other, hence disrupting signaling cascades that help cancer cells evade T cell-mediated cell death. Immune checkpoint inhibitors modulate interactions between tumor cells and cytotoxic T lymphocytes within the tumor environment, which are exhausted in their function.[124] Currently, two immune checkpoint proteins, cytotoxic T lymphocyte-associated 4 (CTLA-4) and programmed cell death protein 1 (PD-1) or its ligand (PD-L1) have been evaluated. They have been found to positively influence cancer treatment outcomes, disease progression-free and/or overall survival, compared with chemotherapy-based treatment.[124,125] CTLA-4 and PD-1 are known to mediate immunological homeostasis by acting as downregulators of T cell activity after pathogen elimination. The FDA has already approved anti-CTLA-4 antibodies (i.e., 'Ipilimumab'), PD1 antibodies (i.e., 'Nivolumab' and 'Pembrolizumab') and PD-L1 antibodies (i.e., 'Atezolizumab'). Since 2011, these have demonstrated remarkable results either alone or in combination with other drugs or surgery for cancer treatment. This has been observed for many malignancies, including melanoma, Hodgkin's lymphoma, bladder, kidney, and/or lung cancer.[125] Many clinical trials involving combinations of these promising targeting agents are currently under investigation in various cancer types (liver, renal, ovarian, HNSCC, and pancreatic cancers) (refer to Table 4). Further approaches to be applied in the field of hematological

Table 4. Summary of checkpoint inhibitor clinical trials for human cancers. Data taken from http://clinicaltrials.gov/.

Drug	Combination	Sponsor	Tumor types	Sample size	Phases	Recruitment Status	Clinical trial ID
Anti-PD1 antibody							
Nivolumab	Tetrahydrouridine	Yogen Saunthararajah	Non Small Cell Lung Cancer	60	II	Recruiting	NCT02664181
	-	National Cancer Institute (NCI)	Ependymoma, Meningioma, Chordoma	180	II	Recruiting	NCT03173950
	TIL infusion	Inge Marie Svane	Metastatic Ovarian Cancer	12	I/II	Recruiting	NCT03287674
	-	Hospital Moinhos de Vento	Prostate Cancer	29	II	Recruiting	NCT03040791
	Denosumab	Australia and New Zealand Melanoma Trials Group	Metastatic Melanoma	72	I/II	Recruiting	NCT03161756
	TAE	Teclison Ltd.	Liver Cancer	40	II	Recruiting	NCT03259867
	Viagenpumatucel-L	Heat Biologics	Non Small Cell Lung Cancer	120	I/II	Recruiting	NCT02439450
	Radiation	Giuseppe Giaccone	Small Cell Lung Cancer	56	I/II	Recruiting	NCT03325816
	Ipilimumab	Bristol-Myers Squibb	Recurrent or Metastatic HNSCC		III	Recruiting	NCT02741570
	Interleukin-2	University of Michigan Cancer Center	Metastatic Clear Cell Renal Cell Cancer	23	I/II	Recruiting	NCT02989714
	Omaveloxolone or Ipilimumab	Reata Pharmaceuticals, Inc.	Melanoma	102	I/II	Recruiting	NCT02259231
Pembrolizumab	Gemcitabine or Cisplatin	Cedars-Sinai Medical Center	Recurrent Platinum-resistant Ovarian Cancer	25	II	Recruiting	NCT02608684
	Idelalisib	Zhonglin Hao	Non Small Cell Lung Cancer	40	I	Recruiting	NCT03257722
	Docetaxel	Medical University of Vienna	Recurrent or Metastatic Head and Neck Cancer	22	I/II	Recruiting	NCT02718820
	INCB001158	Incyte Corporation	Advanced/Metastatic Solid Tumors	346	I/II	Recruiting	NCT02903914
	Vitamin D	Translational Genomics Research Institute	Pancreatic Cancer	24	II	Recruiting	NCT03331562
	B-701	BioClin Therapeutics, Inc.	Advanced or Metastatic Urothelial Cell Carcinoma	74	I/II	Recruiting	NCT03123055
	Methotrexate/ Docetaxel/Cetuximab	Merck Sharp & Dohme Corp.	Recurrent or Metastatic Head and Neck Cancer	495	III	Active, not recruiting	NCT02252042
	Cisplati/Carboplatin/5-FU/Cetuximab	Merck Sharp & Dohme Corp.	Recurrent or Metastatic HNSCC	825	III	Active, not recruiting	NCT02358031
	-	Kindai University	Hepatocellular Carcinoma	50	II	Not yet recruiting	NCT03337841
	-	Biothera	Advanced MelanomaTriple-Negative Breast Cancer	95	II	Recruiting	NCT02981303
	Olaptesed	NOXXON Pharma AG	Colorectal and Pancreatic Cancer	20	I/II	Recruiting	NCT03168139
	Laser Interstitial Thermotherapy	Comprehensive Cancer Center	Recurrent Glioblastoma	34	I/II	Recruiting	NCT03277638

Continued

Table 4. Continued

Drug	Combination	Sponsor	Tumor types	Sample size	Phases	Recruitment Status	Clinical trial ID
Pembrolizumab or Nivolumab	HyperAcute®-Melanoma	NewLink Genetics Corporation	Metastatic Melanoma	100	II	Unknown	NCT02054520
IBI308	Docetaxel	Innovent Biologics (Suzhou) Co., Ltd.	Squamous Cell Lung Carcinoma	266	III	Recruiting	NCT03150875
JS001	-	Shanghai Junshi Bioscience Co., Ltd.	Advanced or Metastatic Bladder Urothelial Carcinoma	370	II	Recruiting	NCT03113266
JS001	-	Shanghai Junshi Bioscience Co., Ltd.	Mucosal Melanoma	220	II	Recruiting	NCT03178123
PD-1 Antibodies	-	University Hospital Heidelberg	Melanoma	40	II	Recruiting	NCT03171064
Anti-PD-L1 antibody							
Atezolizumab	Radiotherapy	Gustave Roussy, Cancer Campus, Grand Paris	Metastatic Tumors	180	II	Recruiting	NCT02992912
	Guadecitabine	University of Southern California	Acute Myeloid Leukemia	72	I/II	Recruiting	NCT02935361
	Atezolizumab	Immune Design	Sarcoma	88	II	Active, not recruiting	NCT02609984
Avelumab	CMB305	Clinique Neuro-Outaouais	Glioblastoma Multiforme of Brain	30	II	Recruiting	NCT03047473
Blocking interaction of PD1 and PDL1							
Durvalumab	Tremelimumab	Samsung Medical Center	Inoperable Esophageal Cancer	40	II	Recruiting	NCT03377400
PDR001	-	Novartis Pharmaceuticals	Advanced Malignancies	318	I/II	Recruiting	NCT02404441
Anti-CTLA-4 antibody							
Ipilimumab	Nivolumab	Olivia Newton-John Cancer Research Institute	Gastrointestinal Cancer and Neuroendocrine Tumors	60	II	Recruiting	NCT02923934
Olaparib	Cediranib	National Cancer Institute (NCI)	Advanced Solid Tumors	421	I/II	Recruiting	NCT02484404
CDK4/6 inhibitor							
Trilaciclib	Atezolizumab	G1 Therapeutics, Inc.	Small Cell Lung Cancer	105	II	Active, not recruiting	NCT03041311
Others							
Enfortumab vedotin		Astellas Pharma Global Development, Inc.	Advanced or Metastatic Urothelial Bladder Cancer	120	II	Recruiting	NCT03219333
PV-10	Dacarbazine	Provectus Biopharmaceuticals, Inc.	Advanced Cutaneous Melanoma	225	III	Recruiting	NCT02288897
Anti-OX40 Antibody PF-04 518 600	Axitinib	University of Southern California	Metastatic Kidney Cancer	104	II	Recruiting	NCT03092856

malignancies involve combining immune checkpoint inhibitors with chimeric antigen receptor T cells (CAR-T cells). In 2017, CD19-targeting CARs T cell therapy was approved by the FDA. The first one being 'Kymriah™,' which was used for ALL treatment in children. This therapy achieved complete remission in 83% of patients with B cell ALL, although 49% of them suffered from strong cytokine release adverse effects.[126] Similarly, 'Yescarta™' is applicable for adult advanced lymphomas. Initial results show that 72% of patients positively responded to this therapy, with 51% even showing complete remission of cancer after a single infusion.[127] Currently, most of the available checkpoint inhibitor trials are in phase I/II clinical trial stages (Table 4). There are a few ongoing phase III clinical trials, which are investigating checkpoint inhibitors in combination with a single agent (i.e 'Docetaxel,' 'Ipilimumab,' Cisplatin, 5-Fu, 'Cetuximab,' etc.) in SCLC and HNSCC (refer to Table 4). A summary of currently ongoing clinical trials of individual, as well as combined immune checkpoint inhibitors (as registered in ClinicalTrials.gov) is provided in Table 4.

Concluding remarks

The way to reach the ultimate goal of precision treatment of cancer, that is the delivery of effective drugs to each individual patient, based on their characterized molecular profiles requires a considerable amount of basic research to understand the fundamentals of cancer heterogeneity. Cancer is an evolutionary complex, dynamic, and genetically heterogeneous disease, with multiple contributing factors and cellular components involved in its initiation, progression, and metastasis. This immense complexity, together with increasing resistance of tumors against currently implemented therapeutic interventions in clinical settings, requires a thorough understanding of cancer evolution at the cellular, genomic, transcriptomic, epigenetic, and proteomic levels.

In addition to conventional tools used to study cancer heterogeneity in bulk tissues, the recent development and constant optimization of more sophisticated sequencing tools at the single-cell level will continue to advance our insight and knowledge into tumor evolutionary origins, unique microenvironments, as well as metastasis. Studying intra-tumor heterogeneity and the spatial orientation of sub-clones within the primary tumor, via novel spatial transcriptomic methods together with simultaneous multiple 'omic'-sequencing, will promote specific drug targeting of individual tumor sub-clones in the near future. Examining the nature of stem-like tumor cells and the transcriptomic mechanisms required to give rise to new tumor populations, will give clarity to the origin of various metastatic disease states. Targeting these stem-like cells could hamper the spread of cancer throughout the body. Being able to longitudinally isolate and sample CTCs will permit non-invasive diagnosis and monitoring, hence enabling highly personalized treatment. Treatment approaches can be constantly modified upon tracking the response and evolution of CTCs throughout the treatment. Finally, treatment resistance can be prevented through more accurate modeling of tumor resistance development to current drugs or radiotherapy. Much work still remains to make these goals a reality, but as single-cell sequencing methods continue to become cheaper, capable of achieving higher coverage, enabling multi-omic analyses, have higher fidelity and the ability to process a greater number of cells at faster rates, there is no doubt that these goals are attainable. Thus, we are coming closer to a promising future with the enhanced ability to generate new personalized therapeutic strategies in our constant fight against cancer.

Acknowledgements

This work was supported in part by the National Natural Science Foundation of China (Grant No. 81770173), and the National Institutes of Health (Grant No. R01 DK100858).

Conflict of interest statement

None declared.

Authors' contributions

Conception development and article design: Xinghua Pan and Jialing Zhang. Acquisition of data: Jialing Zhang and Stephan Stanislaw Späth. Writing and/or revision the manuscript: Jialing Zhang, Stephan Stanislaw Späth, Sadie L. Marjani, Wengeng Zhang and Xinghua Pan.

References

1. Heppner GH, Shekhar M: Tumor Heterogeneity Is Fundamental to the Tumor Ecosystem. *Oncology-Ny* 2014, **28**:780–1.
2. McGranahan N, Swanton C: Biological and Therapeutic Impact of Intratumor Heterogeneity in Cancer Evolution (vol 27, pg 15, 2015). *Cancer Cell* 2015, **28**:141–1.
3. Tellez-Gabriel M, Ory B, Lamoureux F, *et al.* Tumour Heterogeneity: The Key Advantages of Single-Cell Analysis. *Int J Mol Sci* 2016, **17**. pii: E2142.
4. Meacham CE, Morrison SJ: Tumour heterogeneity and cancer cell plasticity. *Nature* 2013, **501**:328–37.
5. Litzenburger UM, Buenrostro JD, Wu B, *et al.* Single-cell epigenomic variability reveals functional cancer heterogeneity. *Genome Biol* 2017, **18**:15.
6. Kreso A, Dick JE: Evolution of the cancer stem cell model. *Cell Stem Cell* 2014, **14**:275–91.
7. Liu L, Liu J, Shao D, *et al.* Comprehensive genomic profiling of lung cancer using a validated panel to explore therapeutic targets in East Asian patients. *Cancer Sci* 2017, **108**: 2487–94.
8. Shackleton M, Quintana E, Fearon ER, *et al.* Heterogeneity in cancer: cancer stem cells versus clonal evolution. *Cell* 2009, **138**:822–9.

9. Shyr D, Liu Q: Next generation sequencing in cancer research and clinical application. *Biol Proced Online* 2013, **15**:4.
10. Gerlinger M, Rowan AJ, Horswell S, *et al*. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. *New Engl J Med* 2012, **366**:883–92.
11. Akbani R, Ng KS, Werner HM, *et al*. A pan-cancer proteomic analysis of The Cancer Genome Atlas (TCGA) project. [abstract]. In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr 5–9; San Diego, CA. Philadelphia (PA): AACR; *Cancer Res* 2014, 74 (19 Suppl): Abstract nr 4262.
12. Lee H, Palm J, Grimes SM, *et al*. The Cancer Genome Atlas Clinical Explorer: a web and mobile interface for identifying clinical-genomic driver associations. *Genome Med* 2015, **7**: 112.
13. Nakagawa H, Fujita M: Whole genome sequencing analysis for cancer genomics and precision medicine. *Cancer Sci* 2018, **109**:513–22.
14. Zhang XY, Marjani SL, Hu ZY, *et al*. Single-Cell Sequencing for Precise Cancer Research: Progress and Prospects. *Cancer Res* 2016, **76**:1305–12.
15. Dalerba P, Kalisky T, Sahoo D, *et al*. Single-cell dissection of transcriptional heterogeneity in human colon tumors. *Nat Biotechnol* 2011, **29**:1120–U1111.
16. Vogelstein B, Papadopoulos N, Velculescu VE, *et al*. Cancer genome landscapes. *Science* 2013, **339**:1546–58.
17. Weinstein JN, Collisson EA, Mills GB, *et al*. Network CGAR: The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet* 2013, **45**:1113–20.
18. Patel JP, Gonen M, Figueroa ME, *et al*. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med* 2012, **366**:1079–89.
19. Shah SP, Roth A, Goya R, *et al*. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature* 2012, **486**:395–9.
20. Cancer Genome Atlas Research Network Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012, **489**:519–25.
21. Koboldt DC, Fulton RS, McLellan MD, *et al*. Comprehensive molecular portraits of human breast tumours. *Nature* 2012, **490**:61–70.
22. Wyatt AW, Mo F, Wang K, *et al*. Heterogeneity in the inter-tumor transcriptome of high risk prostate cancer. *Genome Biol* 2014, **15**: 426.
23. Lohr JG, Stojanov P, Carter SL, *et al*. Widespread Genetic Heterogeneity in Multiple Myeloma: Implications for Targeted Therapy. *Cancer Cell* 2014, **25**:91–101.
24. Network TC: Corrigendum: Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2013, **494**:506.
25. Cancer Genome Atlas Network Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012, **487**:330–7.
26. Li S, Garrett-Bakelman FE, Chung SS, *et al*. Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid leukemia. *Nat Med* 2016, **22**:792–9.
27. Lawrence MS, Stojanov P, Polak P, *et al*. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 2013, **499**:214–8.
28. Kalikaki A, Koutsopoulos A, Trypaki M, *et al*. Comparison of EGFR and K-RAS gene status between primary tumours and corresponding metastases in NSCLC. *Brit J Cancer* 2008, **99**: 923–9.
29. Albanese I, Scibetta AG, Migliavacca M, *et al*. Heterogeneity within and between primary colorectal carcinomas and matched metastases as revealed by analysis of Ki-ras and p53 mutations. *Biochem Bioph Res Commun* 2004, **325**:784–91.
30. Sottoriva A, Kang H, Ma ZC, *et al*. A Big Bang model of human colorectal tumor growth. *Nat Genet* 2015, **47**:209–16.
31. Morris LGT, Riaz N, Desrichard A, *et al*. Pan-cancer analysis of intratumor heterogeneity as a prognostic determinant of survival. *Oncotarget* 2016, **7**:10051–63.
32. Merlo LMF, Pepper JW, Reid BJ, *et al*. Cancer as an evolutionary and ecological process. *Nat Rev Cancer* 2006, **6**:924–35.
33. Korolev KS, Xavier JB, Gore J: Turning ecology and evolution against cancer. *Nat Rev Cancer* 2014, **14**:371–80.
34. Taylor TB, Johnson LJ, Jackson RW, *et al*. First steps in experimental cancer evolution. *Evol Appl* 2013, **6**:535–48.
35. Shain AH, Yeh I, Kovalyshyn I, *et al*. The Genetic Evolution of Melanoma from Precursor Lesions. *New Engl J Med* 2015, **373**:1926–36.
36. Nguyen LV, Pellacani D, Lefort S, *et al*. Barcoding reveals complex clonal dynamics of de novo transformed human mammary cells. *Nature* 2015, **528**:267–71.
37. Sievers CK, Leystra AA, Clipson L, *et al*. Understanding Intratumoral Heterogeneity: Lessons from the Analysis of At-Risk Tissue and Premalignant Lesions in the Colon. *Cancer Prev Res* 2016, **9**:638–41.
38. Dohner H, Estey EH, Amadori S, *et al*. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood* 2010, **115**:453–74.
39. Shin SY, Lee ST, Kim HJ, *et al*. Mutation profiling of 19 candidate genes in acute myeloid leukemia suggests significance of DNMT3A mutations. *Oncotarget* 2016, **7**:54825–37.
40. Ok CY, Patel KP, Garcia-Manero G, *et al*. TP53 mutation characteristics in therapy-related myelodysplastic syndromes and acute myeloid leukemia is similar to de novo diseases. *J Hematol Oncol* 2015, **8**: 45.
41. Ley TJ: Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia (vol 368, pg 2059, 2013). *New Engl J Med* 2013, **369**:98–8.
42. Duncavage EJ, Abel HJ, Szankasi P, *et al*. Targeted next generation sequencing of clinically significant gene mutations and translocations in leukemia. *Modern Pathol* 2012, **25**:795–804.
43. Bohers E, Mareschal S, Bouzelfen A, *et al*. Targetable Activating Mutations are Very Frequent in GCB and ABC Diffuse Large B-Cell Lymphoma. *Gene Chromosome Canc* 2014, **53**:144–53.
44. Jardin F: Next generation sequencing and the management of diffuse large B-cell lymphoma: from whole exome analysis to targeted therapy. *Discov Med* 2014, **18**:51–65.
45. Zhang J, Grubor V, Love CL, *et al*. Genetic heterogeneity of diffuse large B-cell lymphoma. *Proc Natl Acad Sci U S A* 2013, **110**:1398–1403.
46. Compagno M, Lim WK, Grunn A, *et al*. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. *Nature* 2009, **459**:717–21.
47. Gonzalez-Aguilar A, Idbaih A, Boisselier B, *et al*. Recurrent mutations of MYD88 and TBL1XR1 in primary central nervous system lymphomas. *Clin Cancer Res* 2012, **18**: 5203–5211.

48. Araujo LH, Timmers C, Bell EH, *et al*. Genomic Characterization of Non-Small-Cell Lung Cancer in African Americans by Targeted Massively Parallel Sequencing. *J Clin Oncol* 2015, **33**:1966–73.
49. Chen F, Zhang Y, Parra E, *et al*. Multiplatform-based molecular subtypes of non-small-cell lung cancer. *Oncogene* 2017, **36**:1384–93.
50. Lawrence MS, Sougnez C, Lichtenstein L, *et al*. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* 2015, **517**:576–82.
51. Hajek M, Sewell A, Kaech S, *et al*. TRAF3/CYLD mutations identify a distinct subset of human papillomavirus-associated head and neck squamous cell carcinoma. *Cancer* 2017, **123**:1778–90.
52. Zhang J, Cheng H, Yang X, *et al*. Alternative NF-κB pathway activation enhanced by deficient TRAF3 in human papillomavirus (HPV)-associated head and neck cancer. [abstract]. In: *Proceedings of the 105th Annual Meeting of the American Association for Cancer Research*, 2014 Apr 5–9; San Diego, CA. Philadelphia (PA): AACR; *Cancer Res* 2014, 74 (19 Suppl): Abstract 3170.
53. Gatalica Z, Vranic S, Xiu J, *et al*. High microsatellite instability (MSI-H) colorectal carcinoma: a brief review of predictive biomarkers in the era of personalized medicine. *Fam Cancer* 2016, **15**:405–12.
54. Lochhead P, Chan AT, Giovannucci E, *et al*. Progress and Opportunities in Molecular Pathological Epidemiology of Colorectal Premalignant Lesions. *Am J Gastroenterol* 2014, **109**:1205–14.
55. Guglielmini PF, Rossi M, Grosso F, *et al*. Double KRAS and BRAF mutations in colorectal cancer in a single oncologic department series. *J Clin Oncol* 2013, **31** (15 Suppl): Abstract e14657.
56. The Cancer Genome Atlas Network Comprehensive molecular portraits of human breast tumours. *Nature* 2012, **490**:61–70.
57. Dubsky P, Brase JC, Jakesz R, *et al*. The EndoPredict score provides prognostic information on late distant metastases in ER+/HER2- breast cancer patients. *Brit J Cancer* 2013, **109**: 2959–64.
58. Zonno KD, Kaldate RR, Arnell C, *et al*. BRCA1/2 mutation prevalence among triple-negative breast cancer patients from a large commercial testing cohort. *J Clin Oncol* 2013, **31** (15 Suppl): Abstract 1544.
59. Wen WX, Soo JSS, Kwan PY, *et al*. Germline APOBEC3B deletion is associated with breast cancer risk in an Asian multiethnic cohort and with immune cell presentation. *Breast Cancer Res* 2016, **18**: 56.
60. Ellsworth RE, Blackburn HL, Shriver CD, *et al*. Molecular heterogeneity in breast cancer: State of the science and implications for patient care. *Semin Cell Dev Biol* 2017, **64**: 65–72.
61. Seol H, Lee HJ, Choi Y, *et al*. Intratumoral heterogeneity of HER2 gene amplification in breast cancer: its clinicopathological significance. *Modern Pathol* 2012, **25**: 938–48.
62. George E, Kim H, Krepler C, *et al*. A patient-derived-xenograft platform to study BRCA-deficient ovarian cancers. *JCI Insight* 2017, **2**:1–18.
63. Wang WX, Figg WD: Secondary BRCA1 and BRCA2 alterations and acquired chemoresistance. *Cancer Biol Ther* 2008, **7**:1004–5.
64. Lopez-Chavez A, Carter CA, Giaccone G: The role of KRAS mutations in resistance to EGFR inhibition in the treatment of cancer. *Curr Opin Investig Drugs* 2009, **10**:1305–14.
65. Housman G, Byler S, Heerboth S, *et al*. Drug Resistance in Cancer: An Overview. *Cancers* 2014, **6**:1769–92.
66. Baselga J, Campone M, Piccart M, *et al*. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med* 2012, **366**:520–9.
67. Beck JT: Potential role for mammalian target of rapamycin inhibitors as first-line therapy in hormone receptor-positive advanced breast cancer. *Oncotargets Ther* 2015, **8**:3629–38.
68. Uchibori K, Inase N, Araki M, *et al*. Brigatinib combined with anti-EGFR antibody overcomes osimertinib resistance in EGFR-mutated non-small-cell lung cancer. *Nat Commun* 2017, **8**:14768.
69. Li H, Hu HC, Wang R, *et al*. Primary concomitant EGFR T790M mutation predicted worse prognosis in non-small cell lung cancer patients. *Oncotargets Ther* 2014, **7**:513–24.
70. Turke AB, Zejnullahu K, Wu YL, *et al*. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. *Cancer Cell* 2010, **17**:77–88.
71. Flaherty KT, Infante JR, Daud A, *et al*. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med* 2012, **367**:1694–1703.
72. Davies H, Bignell GR, Cox C, *et al*. Mutations of the BRAF gene in human cancer. *Nature* 2002, **417**:949–54.
73. Ascierto PA, McArthur GA, Dreno B, *et al*. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. *Lancet Oncol* 2016, **17**: 1248–60.
74. Valent P: Imatinib-resistant chronic myeloid leukemia (CML): Current concepts on pathogenesis and new emerging pharmacologic approaches. *Biologics* 2007, **1**:433–48.
75. Holleman A, Cheok MH, den Boer ML, *et al*. Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. *New Engl J Med* 2004, **351**: 533–42.
76. McComb S, Aguade-Gorgorio J, Harder L, *et al*. Activation of concurrent apoptosis and necroptosis by SMAC mimetics for the treatment of refractory and relapsed ALL. *Sci Transl Med* 2016, **8**:339ra70.
77. Nugues AL, El Bouazzati H, Hetuin D, *et al*. RIP3 is downregulated in human myeloid leukemia cells and modulates apoptosis and caspase-mediated p65/RelA cleavage. *Cell Death Dis* 2014, **5**:e1384.
78. Ding L, Ley TJ, Larson DE, *et al*. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature* 2012, **481**:506–10.
79. Dhillon KK, Swisher EM, Taniguchi T: Secondary mutations of BRCA1/2 and drug resistance. *Cancer Sci* 2011, **102**:663–9.
80. Van Cutsem E, Tabernero J, Lakomy R, *et al*. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J Clin Oncol* 2012, **30**:3499–3506.
81. Leto SM, Trusolino L: Primary and acquired resistance to EGFR-targeted therapies in colorectal cancer: impact on future treatment strategies. *J Mol Med (Berl)* 2014, **92**:709–22.
82. Smith DH, Fiehn AM, Fogh L, *et al*. Measuring ERCC1 protein expression in cancer specimens: validation of a novel antibody. *Sci Rep* 2014, **4**:4313.

83. Van Emburgh BO, Arena S, Siravegna G, *et al*. Acquired RAS or EGFR mutations and duration of response to EGFR blockade in colorectal cancer. *Nat Commun* 2016, **7**:13665.
84. Sartore-Bianchi A, Trusolino L, Martino C: Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial (vol 17, pg 738, 2016). *Lancet Oncol* 2016, **17**:E420–0.
85. Friedman R: Drug resistance in cancer: molecular evolution and compensatory proliferation. *Oncotarget* 2016, **7**:11746–55.
86. Ellis LM, Hicklin DJ: Resistance to Targeted Therapies: Refining Anticancer Therapy in the Era of Molecular Oncology. *Clin Cancer Res* 2009, **15**:7471–8.
87. Izar B, Rotow J, Gainor J, *et al*. Pharmacokinetics, clinical indications, and resistance mechanisms in molecular targeted therapies in cancer. *Pharmacol Rev* 2013, **65**:1351–95.
88. Navin N, Kendall J, Troge J, *et al*. Tumour evolution inferred by single-cell sequencing. *Nature* 2011, **472**:90–4.
89. Eberwine J, Yeh H, Miyashiro K, *et al*. Analysis of gene expression in single live neurons. *Proc Natl Acad Sci U S A* 1992, **89**:3010–4.
90. Gawad C, Koh W, Quake SR: Single-cell genome sequencing: current state of the science. *Nat Rev Genet* 2016, **17**:175–88.
91. Patel AP, Tirosh I, Trombetta JJ, *et al*. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science* 2014, **344**:1396–1401.
92. Jordan NV, Bardia A, Wittner BS, *et al*. HER2 expression identifies dynamic functional states within circulating breast cancer cells. *Nature* 2016, **537**:102–6.
93. Gorner K, Bachmann J, Schiemann M, *et al*. Circulating Tumour Cells: Analysis of Single Cells Isolated from Ductal Pancreatic Adenocarcinoma Patients. *Tumor Biol* 2010, **31**:S81–1.
94. Chen XX, Bai F: Single-cell analyses of circulating tumor cells. *Cancer Biol Med* 2015, **12**:184–92.
95. Ni XH, Zhuo ML, Su Z, *et al*. Single Cell Genomic Analyses of Circulating Tumor Cells from Lung Cancer Patients. *J Thorac Oncol* 2013, **8**:S493–3.
96. Wu CP, Wu P, Zhao HF, *et al*. Clinical Applications of and Challenges in Single-Cell Analysis of Circulating Tumor Cells. *DNA Cell Biol* 2018, **37**:78–89.
97. Taenzer A, Alix-Panabieres C, Wikman H, *et al*. Circulating tumor-derived biomarkers in lung cancer. *J Thorac Dis* 2012, **4**:448–9.
98. Van Loo P, Voet T: Single cell analysis of cancer genomes. *Curr Opin Genet Dev* 2014, **24**:82–91.
99. Fallahi-Sichani M, Moerke NJ, Niepel M, *et al*. Systematic analysis of BRAF(V600E) melanomas reveals a role for JNK/c-Jun pathway in adaptive resistance to drug-induced apoptosis. *Mol Syst Biol* 2015, **11**: 797.
100. Galletti G, Portella L, Tagawa ST, *et al*. Circulating Tumor Cells in Prostate Cancer Diagnosis and Monitoring: An Appraisal of Clinical Potential. *Mol Diagn Ther* 2014, **18**:389–402.
101. Miyamoto DT, Zheng Y, Wittner BS, *et al*. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. *Science* 2015, **349**:1351–6.
102. Janiszewska M, Liu L, Almendro V, *et al*. In situ single-cell analysis identifies heterogeneity for PIK3CA mutation and HER2 amplification in HER2-positive breast cancer. *Nat Genet* 2015, **47**:1212.
103. Porta C, Paglino C, Mosca A: Targeting PI3K/Akt/mTOR Signaling in Cancer. *Front Oncol* 2014, **4**:64.
104. Wang X, Ding J, Meng LH: PI3K isoform-selective inhibitors: next-generation targeted cancer therapies. *Acta Pharmacol Sin* 2015, **36**:1170–6.
105. Guarinos C, Juarez M, Egoavil C, *et al*. Prevalence and characteristics of MUTYH-associated polyposis in patients with multiple adenomatous and serrated polyps. *Clin Cancer Res* 2014, **20**:1158–68.
106. Markham A: Copanlisib: First Global Approval. *Drugs* 2017, **77**:2057–62.
107. Massacesi C, Di Tomaso E, Urban P, *et al*. PI3K inhibitors as new cancer therapeutics: implications for clinical trial design. *Oncotargets Ther* 2016, **9**:203–10.
108. Brufsky AM: Current Approaches and Emerging Directions in HER2-resistant Breast Cancer. *Breast Cancer (Auckl)* 2014, **8**:109–18.
109. Van Waes C, Musbahi O: Genomics and advances towards precision medicine for head and neck squamous cell carcinoma. *Laryngoscope Investig Otolaryngol* 2017, **2**:310–9.
110. Mendoza MC, Er EE, Blenis J: The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends Biochem Sci* 2011, **36**:320–8.
111. Tripathy D, Bardia A, Sellers WR: Ribociclib (LEE011): Mechanism of Action and Clinical Impact of This Selective Cyclin-Dependent Kinase 4/6 Inhibitor in Various Solid Tumors. *Clinical Cancer Research* 2017, **23**: 3251–62.
112. Eroglu Z, Ribas A: Combination therapy with BRAF and MEK inhibitors for melanoma: latest evidence and place in therapy. *Ther Adv Med Oncol* 2016, **8**:48–56.
113. Karin M: Nuclear factor-kappaB in cancer development and progression. *Nature* 2006, **441**:431–6.
114. Park MH, Hong JT: Roles of NF-kappaB in Cancer and Inflammatory Diseases and Their Therapeutic Approaches. *Cells* 2016, **5**:15.
115. Cai Q, Tu M, Xu-Monette ZY, *et al*. NF-kappaB p50 activation associated with immune dysregulation confers poorer survival for diffuse large B-cell lymphoma patients with wild-type p53. *Mod Pathol* 2017, **30**:854–76.
116. Hunter JE, Butterworth JA, Zhao B, *et al*. The NF-kappaB subunit c-Rel regulates Bach2 tumour suppressor expression in B-cell lymphoma. *Oncogene* 2016, **35**: 3476–84.
117. Tabruyn SP, Griffioen AW: NF-kappa B: a new player in angiostatic therapy. *Angiogenesis* 2008, **11**:101–6.
118. Prabhu L, Mundade R, Korc M, *et al*. Critical role of NF-kappaB in pancreatic cancer. *Oncotarget* 2014, **5**: 10969–75.
119. Gupta SC, Sundaram C, Reuter S, *et al*. Inhibiting NF-kappaB activation by small molecules as a therapeutic strategy. *Biochim Biophys Acta* 2010, **1799**:775–87.
120. Xue W, Meylan E, Oliver TG, *et al*. Response and resistance to NF-kappaB inhibitors in mouse models of lung adenocarcinoma. *Cancer Discov* 2011, **1**:236–47.
121. Godwin P, Baird AM, Heavey S, *et al*. Targeting nuclear factor-kappa B to overcome resistance to chemotherapy. *Front Oncol* 2013, **3**:120.
122. Liu Y, Zeng G: Cancer and innate immune system interactions: translational potentials for cancer immunotherapy. *J Immunother* 2012, **35**:299–308.
123. Vinay DS, Ryan EP, Pawelec G, *et al*. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Semin Cancer Biol* 2015, **35** S185–98.

124. Azoury SC, Straughan DM, Shukla V: Immune Checkpoint Inhibitors for Cancer Therapy: Clinical Efficacy and Safety. *Curr Cancer Drug Targets* 2015, **15**:452–62.
125. Dine J, Gordon R, Shames Y, *et al.* Immune Checkpoint Inhibitors: An Innovation in Immunotherapy for the Treatment and Management of Patients with Cancer. *Asia Pac J Oncol Nurs* 2017, **4**:127–35.
126. Tasian SK, Gardner RA: CD19-redirected chimeric antigen receptor-modified T cells: a promising immunotherapy for children and adults with B-cell acute lymphoblastic leukemia (ALL). *Ther Adv Hematol* 2015, **6**:228–41.
127. Makita S, Yoshimura K, Tobinai K: Clinical development of anti-CD19 chimeric antigen receptor T-cell therapy for B-cell non-Hodgkin lymphoma. *Cancer Sci* 2017, **108**:1109–18.