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ΑΠΟ ΤΗΝ ΟΓΚΟΓΕΝΕΣΗ ΕΩΣ ΤΗ ΘΕΡΑΠΕΙΑ

MASTER THESIS

INVESTIGATION OF SIGNALING PATHWAYS IMPLICATED IN CANCER DEVELOPMENT

SUPERVISOR: RESEARCH DIRECTOR, VASSILIS ZOUMPOURLIS

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1140025
ATHENS, 2022

Η παρούσα διπλωματική εργασία εκπονήθηκε στο πλαίσιο σπουδών για την απόκτηση του Μεταπτυχιακού Διπλώματος Ειδίκευσης που απονέμει το Τμήμα Ιατρικής του Πανεπιστημίου Κρήτης, σε συνεργασία με το Ινστιτούτο Χημικής Βιολογίας του Εθνικού Ιδρύματος Ερευνών.

Εγκρίθηκε από τη κάτωθι τριμελή εξεταστική επιτροπή:

ΤΡΙΜΕΛΗΣ ΕΠΙΤΡΟΠΗ

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Στους διστακτικούς, που τελικά βρίσκουν τον τρόπο.

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Abstract

Cancer is the second leading cause of death globally. A main hallmark in cancer is the functional deregulation of crucial molecular pathways via driver gene mutations that lead to abnormal gene expression, giving cells a selective growth advantage. Molecular analysis on tissues originated from a wide range of anatomical areas has shown that mutations in different members of several pathways are implicated in different cancer types. In the last decades, significant efforts have been made in order to incorporate this knowledge into the daily medical practice, providing substantial insights towards clinical diagnosis and personalized therapies. However, since there is still a strong need for more effective drug development, a deep understanding of the involved signaling mechanisms and the interconnections between these pathways, is highly anticipated. Here, a systemic analysis on cancer patients of the Pan-Cancer Atlas project was performed, with the aim to select the ten most highly mutated signaling pathways (p53, RTK-RAS, lipids metabolism, PI-3-Kinase/Akt, ubiquitination, b-catenin/Wnt, Notch, cell cycle, homology directed repair (HDR) and splicing) and to provide a detailed description of each pathway's main deregulation mechanisms, along with corresponding therapeutic applications currently being developed or applied.

Keywords

Molecular oncology, precision medicine, cancer patients, mutations, clinical implementation, clinical studies

ΠΕΡΙΛΗΨΗ

Ο καρκίνος αποτελεί την δεύτερη αιτία θανάτου παγκοσμίως. Ένα από τα χαρακτηριστικά γνωρίσματα του καρκίνου είναι η λειτουργική απορρύθμιση κομβικών μοριακών μονοπατιών μέσω οδηγών μεταλλάξεων που συνεπάγονται μη φυσιολογική γονιδιακή έκφραση, προσδίδοντας στα κύτταρα ένα εκλεκτικό αυξητικό πλεονέκτημα. Μοριακή ανάλυση ιστών προερχόμενων από μια ποικιλία ανατομικών περιοχών, έχει δείξει πως μεταλλάξεις σε διαφορετικά μέλη των μονοπατιών αυτών, εμπλέκονται σε διαφορετικούς τύπους καρκίνου. Τις τελευταίες δεκαετίες, έχει γίνει προσπάθεια ενσωμάτωσης αυτής της γνώσης στην καθημερινή ιατρική πράξη, παρέχοντας ουσιαστικές προόδους τόσο σε επίπεδο κλινικής διάγνωσης, όσο και σε επίπεδο εξατομικευμένων θεραπειών. Παρ' όλα αυτά, η παραμένουσα ανάγκη ανάπτυξης αποτελεσματικότερων θεραπειών, καθιστά επιτακτική την βαθιά κατανόηση τόσο των εμπλεκόμενων σηματοδοτικών μηχανισμών, όσο και των μεταξύ τους διασυνδέσεων. Στην παρούσα εργασία διενεργήθηκε συστημική ανάλυση σε καρκινοπαθείς του προγράμματος Pan-Cancer Atlas, προκειμένου να επιλεχθούν τα δέκα συχνότερα μεταλλαγμένα σηματοδοτικά μονοπάτια (p53, RTK-RAS, μεταβολισμός λιπιδίων, PI-3-Kinase/Akt, ουβικιτίνωση, b-catenin/Wnt, Notch, κυτταρικός κύκλος, επιδιόρθωση μέσω ομόλογου ανασυνδυασμού (HDR) και μάτισμα). Παράλληλα παρέχεται μία λεπτομερής περιγραφή των κυριότερων μηχανισμών διατάραξης κάθε μονοπατιού, ενώ γίνεται λόγος και για τις αντίστοιχες εδραιωμένες αλλά και ορισμένες αναπτυσσόμενες θεραπευτικές εφαρμογές.

ΛΕΞΕΙΣ-ΚΛΕΙΔΙΑ:

Μοριακή ογκολογία, ιατρική ακριβείας, ασθενείς με καρκίνο, μεταλλάξεις, κλινική εφαρμογή, κλινικές μελέτες

Scope

The aim of this thesis is to review the current knowledge of the most frequently dysregulated cancer-related signaling cascades and to address the attractive perspectives arising from ongoing experimental studies in the context of clinically implemented personalized medicine.

1. INTRODUCTION

1.1. Personalized therapeutic strategies require understanding of the underlying signaling pathways' mechanisms

In 2020, over 19 million people were diagnosed with cancer and cancer was the cause of death for approximately 10 million (Sung *et al.*, 2021). These rates indicate that cancer constitutes one of the most critical health threats and that there is an imperative need for new more effective therapies. In order to achieve the latter, it is critical to comprehend the main routes whereby different cancer types arise and progress. As cancer is a genetic disease arising from a variety of causative mutations, its effective treatment requires personalized molecular approaches. A robust impetus towards this direction was given especially after the completion of the Human Genome Project (HGP) in 2003 (Goodwin *et al.*, 2016; Carrasco-Ramiro *et al.*, 2017). Several individualized anti-cancer therapies have been developed and applied since then (Imai *et al.*, 2006; Sharma *et al.*, 2015; O'Connor *et al.*, 2015).

Cancer can be regarded as a collection of diseases which all share a common feature; the deregulation of key signaling cascades that leads to uncontrolled cellular proliferation (Parui *et al.*, 2019). Malignancies arise from alterations in the DNA sequence of genes, as well as from epigenetic changes (Sever *et al.*, 2015; Okugawa *et al.*, 2015; Martincorena *et al.*, 2015; Baylin *et al.*, 2016; Feinberg *et al.*, 2016). Such alterations lead to the activation of oncogenes or to the inactivation of tumor-suppressor genes (Lodish *et al.*, 2000). In either cases, signal transduction pathways in which these genes are involved, become deregulated, thus leading to cellular stress-related outcomes like the evasion of growth suppressors, resistance to cell death (apoptosis), cell cycle deregulation, as well as increased invasive and metastatic potential (Hanahan *et al.*, 2011).

Traditionally, the most widely used way to discriminate cancer types from each other is by their anatomical site. However, recently, it has become clear that the underlying dysregulated pathways are able to provide vital information capable of separating and clustering cancer types more thoroughly (Sanchez-Vega *et al.*, 2018). Several signaling cascades, including the b-catenin/Wnt, RTK/RAS, p53 and Sonic Hedgehog (SHH) pathways, are cancer-related due to genetic variations that often occur in the respective genes (Vogelstein *et al.*, 2004; Dempke *et al.*, 2017). Identification of mutations in genes implicated in such pathways, reveals the causative driver events of carcinogenesis (Pon *et al.*, 2015) and therefore constitutes a topic of paramount importance in precision medicine and for the development of efficient tailored-made drug therapies targeting each cancer type at the molecular level.

Personalized cancer therapy is a treatment strategy that takes into account the molecular profile of patients in order to segregate them into groups which are more likely to benefit from different therapeutic approaches (Johnson *et al.*, 2015). Especially during the past fifteen years, numerous targeted therapies have been approved, extending the survival rates of cancer. For instance, administration of monoclonal antibodies such as cetuximab, that is used in colorectal cancers overexpressing the *EGFR* gene (Saltz *et al.*, 2004), as well as tyrosine kinase inhibitors like crizotinib, which is used for non-small cell lung cancers (NSCLC) positive for *ALK* fusions (Shaw *et al.*, 2013), have been dynamically integrated into oncology practice. Beyond pharmacogenetic approaches, there is an increasing interest for gene editing approaches and especially for the clustered regularly interspaced short palindrome repeats (CRISPR)/Cas9 system, which is being tested in multiple clinical trials for its ability to genetically modify immune cells ex vivo (e.g. PD-1 knockout T-cells), thus enhancing the anti-cancer immune response when infused back to the patient (Zhan *et al.*, 2019; Khalaf *et al.*, 2020). Additional studies and clinical implementations of CRISPR/Cas9 are highly anticipated as a means to pave the way for the development of more effective anti-cancer therapies that target specific genetic alterations, thereby restoring their function.

1.2. Systemic analysis determines prevalent mutated carcinogenic pathways

The aim of this study is to underscore the importance of the underlying mechanisms in crucial carcinogenic signaling pathways. To this purpose, attention was focused on the signaling pathways that are most frequently found mutated in cancers. In particular, cancer-related pathways previously reported by Pan-Cancer Atlas project studies have been investigated (Sanchez-Vega *et al.*, 2018; Knijnenburg *et al.*, 2018; Seiler *et al.*, 2018; Ge *et al.*, 2018; Peng *et al.*, 2018; Thorsson *et al.*, 2018) and the frequencies of each driver mutation to the corresponding pathways have been calculated. For this analysis publicly available data of 10,439 tumor samples from 32 TCGA Pan-Cancer Atlas studies (Cerami *et al.*, 2012) were used. The results suggest that the top ten rated pathways may be sorted in the following descending order of driver mutational frequency: p53, RTK-RAS, lipids metabolism, PI-3-Kinase/Akt, ubiquitination, b-catenin/Wnt, Notch, cell cycle, homology directed repair (HDR) and splicing.

From TCGA Pan-Cancer Atlas publications (available in https://www.cell.com/pb-assets/consortium/pancanceratlas/pancani3/index.html) the available pathways' gene lists (3,004 genes in total) were collected. Then, only genes belonging to the OncoKB cancer gene list (340 genes) - as it had been formed until February 2021 – were filtered. Using the Onco Query Language (OQL) provided by cBioPortal, a search was held specifically for

somatic driver mutations in these genes in 10,439 mutationally profiled tumor samples (10,066 primary, 364 metastatic and 9 samples of recurrent origin) of 32 TCGA Pan-Cancer Atlas studies. In order to avoid misleading results, the samples of metastatic and recurrent origin were separated from those of primary origin and the attention was focused on the latter, which represented the vast majority in the final dataset (7,915 out of 8,276 samples). Data were extracted from the cBioPortal for Cancer Genomics open-access resource. Somatic driver mutations were identified for 204 out of the 340 cancer genes in this particular dataset, so the interest was directed to the pathways these genes participate in. Data management, calculations and graph construction were performed using Spyder IDE, a scientific python development environment.

2. MAIN BODY

Numerous TCGA Pan-Cancer Atlas efforts have been made to elucidate the genomic background of cancer, each focusing on the deregulation of a different physiological cellular procedure (Sanchez-Vega et al., 2018). Each one of these procedures involves a number of signaling pathways that, in turn, contain genes with a distinct tumorigenic potential (**Figure 1**). Furthermore, the mutational data analysis held here, has shown that most cancers are characterized by the deregulation of a group of pathways, rather a single signaling pathway, but in a distinct pattern from each other, while in some tumor types, a stage-dependent pathway perturbation also seems to apply (**Figure 2**). For the needs of this review, the findings on the ten most frequently mutated signaling pathways are described. In the next sections, a description of how specific genetic alterations/mutations on a given pathway's encoding genes lead to signaling deregulation during carcinogenesis and/or cancer progression is being held. For each pathway, a brief description of its link to cancer is provided, along with a description of the most highly mutated involved genes, followed by their percentile frequencies, as calculated in this analysis. In addition, some related targeted therapies that have been approved by the Food and Drug Administration (FDA) and are currently being integrated into clinical practice, as well as some emerging therapies which are currently being tested in clinical trials are listed.

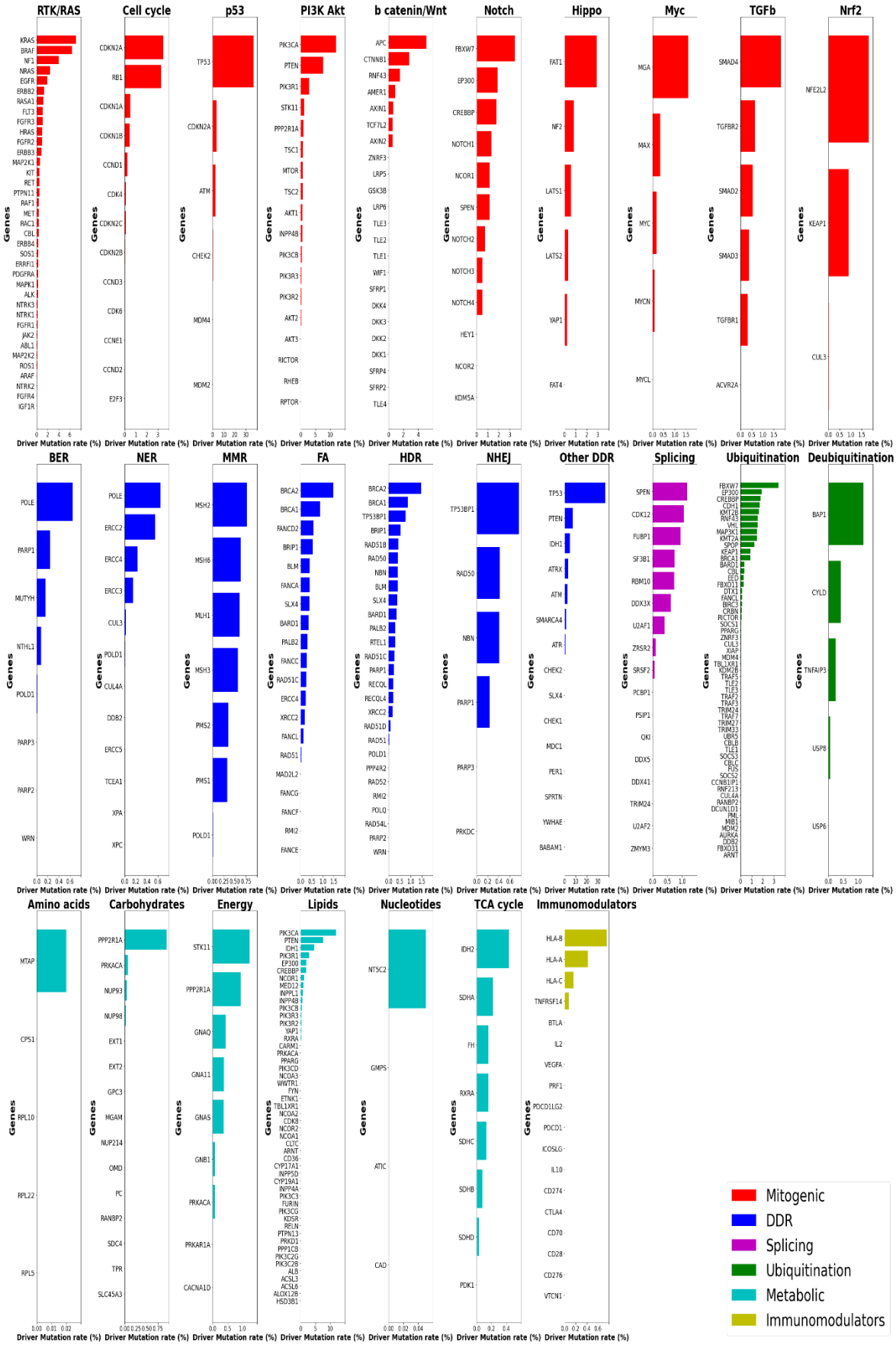

Figure 1. Somatic driver mutational frequency of 204 cancer genes involved in 27 signaling pathways implicated in 6 major cellular procedures. For the calculations, mutational data from 10,439 tumor samples were examined. Percentages refer to the total number of samples examined (10,439).

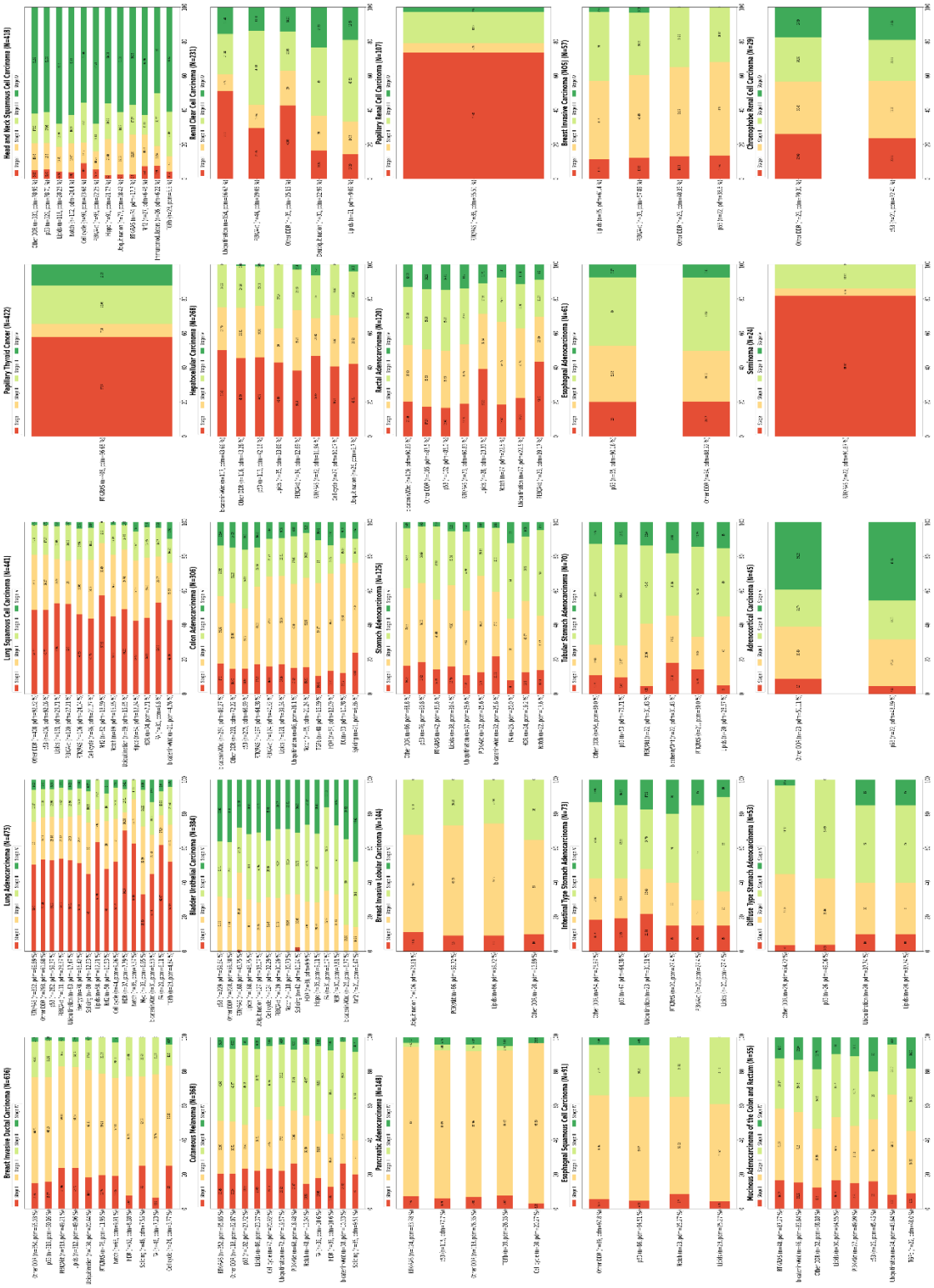

Figure 2. Crucial signaling pathways implicated in 25 cancer types/subtypes per disease stage. For this analysis, 5,283 tumor samples with available mutational profile and disease stage were examined. Here, only signaling pathways altered in at least 20 of the examined samples of each cancer type/subtype are shown. N: number of samples examined; n: number of samples that harbor somatic driver mutations in each signaling pathway; pdm: proportion of samples of a particular cancer type/subtype that harbor at least one somatic driver mutation in genes of a particular signaling pathway.

2.1. The p53 pathway

The importance of *TP53* gene malfunction in cancer development becomes perceived by the fact that it occupies the first place in the ranking of genes found to be most frequently genetically altered in cancer (Levine *et al.*, 2009; Aubrey *et al.*, 2016). The transcription factor p53 and the other components of p53 pathway function cooperatively to ensure that the cell will respond effectively against a variety of stress signals that threaten the DNA replication fidelity. In order to do so, these effectors trigger cell cycle arrest, senescence or apoptosis (Oren *et al.*, 2003; Harris *et al.*, 2005). Furthermore, except for determining cell fate after DNA damage, p53 pathway is also implicated in many other cellular procedures such as the preservation of genetic stability, the inhibition of blood vessel formation, the regulation of metabolism, while it has antioxidant properties (Vogelstein *et al.*, 2000; Jones *et al.*, 2005; Sablina *et al.*, 2005; Labuschagne *et al.*, 2018).

Actually, there is only a small number of cancer types / subtypes where p53 pathway is likely not perturbed (e.g. Uveal Melanoma) (**Figure 3**). In the majority of cancer types, more than 30 % of the samples examined harbored driver mutations in at least one key gene of this pathway, while in some cases this percentage exceeded 90 % (e.g. Esophageal Squamous Cell Carcinoma). Although the number of cancer patients with a gene variant in this signaling cascade is extended, however there are only a few different genes affected. Among those patients, 93.66 % had a driver mutation in *TP53*, whereas *CDKN2A* and *ATM* genes were affected in 7.71 % and 6.27 % respectively, dictating the simultaneous presence of some of these aberrant variants in a number of cases.

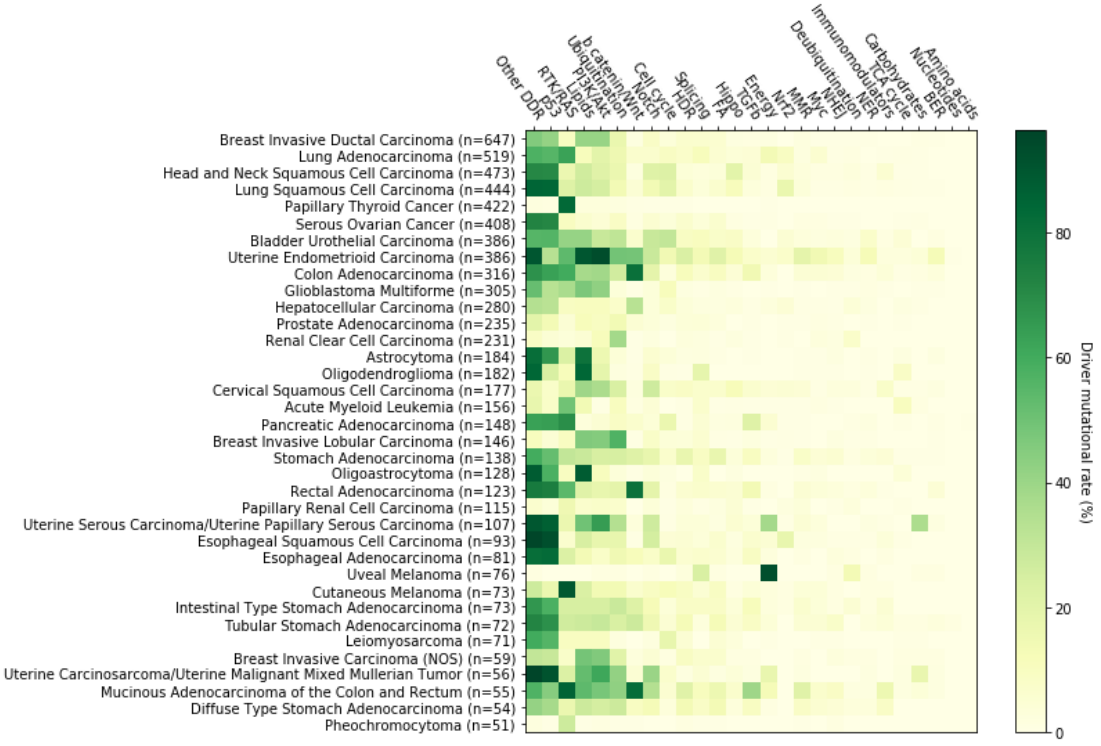

Figure 3. Alteration rate of 27 signaling pathways across 36 cancer types/subtypes. For this analysis, 10,066 tumor samples of primary origin and with available mutational profiles were examined for the presence of somatic driver mutations in the 204 genes of interest. Here, only cancer types/subtypes with more than 50 analyzed samples are shown. From left to right, signaling pathways are displayed in descending order of total mutational frequency. n: number of samples examined.

There is an enormous variety of *TP53* driver mutations in cancer. These mutations can be located in virtually any region of the gene, thus affecting the ability of the p53 protein to appropriately interact with either its protein effectors or the DNA. According to this analysis, arginine residues of positions 273, 248 and 175, which all belong to the DNA-binding domain (Muller *et al.*, 2013), are the most frequently substituted, representing 7.06 %, 5.87 % and 4.41 % of patients that have an affected *TP53* gene. R273 variants have been associated with an increased proliferative and invasive potential, which in the case of R273H, is exerted by the inhibition of tumor suppressor *KLF6* (Li *et al.*, 2014; Sun *et al.*, 2020). Similar effects seem to occur when a R248 mutant is present, but this time, this is attributed to the robust binding of certain variants – mainly the mutp53^{R248Q} - to STAT3 protein and the subsequent strong activation of the latter (Schulz-Heddergott *et al.*, 2018; Klemke *et al.*, 2021). In the case of R175H variant, a radical change in p53 conformation triggers the activation of a new gene panel which results, among other events, to elevated c-Met protein levels that are capable of inducing tumor invasion (Yu *et al.*, 2012; Grugan *et al.*, 2013).

In addition to missense and other mutation types, the *CDKN2A* gene is frequently affected by non-sense mutations, as it was found that R80*, R58*, W110*, E120*, Y44* and

E88* together constitute almost 1/3 of cancer cases that harbor a defective *CDKN2A* gene. Such alterations force the protein $p16^{INK4a}$ to lose its Cdk-binding ability, thereby being unable to prevent the G1/S transition and shutting down one of the cell cycle's checkpoints (Parry *et al.*, 1996; Rutter *et al.*, 2003; Kannengiesser *et al.*, 2009). From the rest 2/3 of cases, an aberrant splicing involving the D153 residue occurs as often as in 5.16 % of all *CDKN2A*-affected cancer patients. Changes in this position may inactivate both *CDKN2A* gene products, i.e. $p16^{INK4a}$ and $p14^{ARF}$, leading to loss of cell cycle control via both pRB and p53 inactivation (Rutter *et al.*, 2003).

In this dataset, non-sense mutations leading to premature truncated forms of the *ATM* protein product, represented more than one third of all *ATM* mutated cases, with R250* being the main representative. In such cases, the final gene product loses its functionality, either partially or completely, leaving cell without one of its main DNA damage sensors and thereby vulnerable to malignant transformation (Easton *et al.*, 2015). The current analysis has also revealed that R337C/H are the most common missense mutations of the *ATM* gene in cancer, accounting together for 7.94 % of all *ATM* variant carriers. Although these substitutions have previously been identified by sequencing (Griffith *et al.*, 2018; Nahar *et al.*, 2018), their functional impact has not been elucidated yet (Jette *et al.*, 2020).

As expected, the dynamic presence of these genes on the mutational cancer map, has made the p53 pathway – and especially the *TP53* component – an attractive therapeutic target. Among a plethora of p53-targeting strategies, the most promising fall into one of the following categories: restoring the function of aberrant p53 protein or impeding the interaction between wt-p53 and its main negative regulator, MDM2 protein (Levine *et al.*, 2009; Duffy *et al.*, 2020; Zhu *et al.*, 2020). In this context, APR-246, a mutant-p53 conformation resetting agent, is probably the most promising compound (Bykov *et al.*, 2018; Duffy *et al.*, 2020) and the only therapy of this category that is currently being tested in a phase III clinical trial (NCT03745716); on the other hand, a phase III trial of idasanutlin (NCT02545283), another emerging MDM2 inhibitor, was recently terminated due to low efficacy. The results of such clinical trials, as well as the efforts to improve the properties of the relevant compounds are highly anticipated.

Apart from p53, there are four FDA approved anticancer drugs targeting *CDKN2A* signaling: abemaciclib (Kim *et al.*, 2017), palbociclib (Dhillon *et al.*, 2015), ribociclib (Syed *et al.*, 2017) and trilaciclib (Dhillon *et al.*, 2021). All these therapeutic agents act as CDK4/6 inhibitors and therefore, their administration may be beneficial in cases of an inactivated

CDKN2A gene (Gopalan *et al.*, 2014; Young *et al.*, 2014; Su *et al.*, 2019). The rationale behind *ATM* deficient-related therapeutic approaches is completely different from those discussed so far; instead of trying to restore the gene function, at this case the goal is to cause synthetic lethality. There is evidence that this is feasible by further weakening DNA repair mechanisms via administration of appropriate inhibitors, mainly PARP and ATR inhibitors, either alone or in combination (Mateo *et al.*, 2015; Schmitt *et al.*, 2017; Lloyd *et al.*, 2020). Ongoing clinical trials may show whether the promising preclinical results can be translated into clinical practice.

2.2. The RTK-RAS pathway

RTK-RAS is probably the most thoroughly studied cancer-related signaling pathway. Its involvement in a multitude of crucial physiological processes, such as cell growth, proliferation, differentiation, angiogenesis, integrin signaling and cell migration (Downward *et al.*, 2003; Kinbara *et al.*, 2003; Molina *et al.*, 2006; Castellano *et al.*, 2016; Imperial *et al.*, 2019), among many others, makes it clear that deregulation of this pathway can facilitate both tumor initiation and tumor progression.

Indeed, this analysis showed that in 12 out of 36 cancer types and subtypes examined, more than 30 % of patients beared driver mutations in RTK-RAS pathway's genes, with this percentage climbing above 80 % in Papillary Thyroid Cancer, Cutaneous Melanoma and Mucinous Adenocarcinoma of the Colon and Rectum. On the other hand, as with the p53 pathway, no Uveal Melanoma patient was affected (**Figure 3**). Regarding the involved genes, *KRAS*, *BRAF* and *NF1* were found as the predominantly mutated ones, being altered in 26.05 %, 17.06 % and 13.06 % of RTK-RAS perturbed cases respectively, while alterations in all cancer-related receptor tyrosine kinase-encoding genes of this pathway (in descending order of mutational frequency: *EGFR*, *ERBB2*, *FGFR3*, *FLT3*, *FGFR2*, *ERBB3*, *RET*, *KIT*, *MET*, *ERBB4*, *PDGFRA*, *ALK*, *FGFR1*, *NTRK3*, *NTRK1*, *ROS1*, *NTRK2*, *IGF1R* and *FGFR4*), represented 33.2 % of all carriers.

As it was ascertained in this cohort, *KRAS* is almost exclusively changed by missense mutations and this happens in a selective way; variants involving residues G12, G13, Q61 and A146 correspond to more than 95 % of all cases (75.41 %, 11.68 %, 5.84 % and 3.67 % respectively). Codon 12, 13 and 61 mutants decrease the GTP-hydrolysis rate, with the first and the latter also being capable of increasing the rate of GDP-exchange (Smith *et al.*, 2013; Cook *et al.*, 2021), while codon 146 mutants act mainly through the second mechanism (Feig *et al.*, 1988; Poulin *et al.*, 2019). Both mechanisms lead to increased levels of the activated GTP-bound form of KRAS protein, resulting in

uncontrolled mitogenic processes via a constitutively active signal transduction (Waters *et al.*, 2018; Cook *et al.*, 2021).

By far the most prevalent mutated codon of *BRAF* gene is the 600th, being involved in 81.74 % of all *BRAF*-mutated primary tumors in this dataset, while rearrangement of *BRAF* with a variety of genes seemed to occur in only 4.36 % of these samples. In the majority of cases where a V600 variant exists, valine residue is substituted by glutamic acid. V600E mutation enhances serine/threonine kinase activity of BRAF protein by 500 times and abrogates the signaling cascade's dependency to extracellular signals, creating a vigorous signaling toward cell growth and proliferation and therefore oncogenic transformation (Davies *et al.*, 2002; Cantwell-Dorris *et al.*, 2011; Falini *et al.*, 2016).

In contrast to *BRAF*, there is no hotspot position being altered in *NF1* gene. This becomes clear from the most common somatic mutation of *NF1*, R2450*, which barely exceeds 3 % of all identified variants in this cohort. Non-sense mutations, frameshift deletions and splice-site variants are the most frequent mutation types for this gene in cancer, accounting for 39.91 %, 20.83 % and 15.79 % of all identified variants respectively. Regardless of the mutation type, in the majority of *NF1*-affected cases, this leads to the loss of neurofibromin's GTPase-activating function, resulting in sustainably high GTP-bound levels of RAS proteins and thereby in a continuous tumor-promoting signaling process via RTK-RAS and PI3K/AKT pathways' hyperactivation (Ding *et al.*, 2008; Upadhyaya *et al.*, 2008; Brems *et al.*, 2009; Hodis *et al.*, 2012).

In regard to the development of targeted anti-cancer therapies, RTK-RAS is the most extensively utilized signaling pathway. Indicatively, 64 targeted therapies have already been approved by the FDA, regarding 18 out of the 37 pathway's genes for which driver mutations were identified in the current dataset (**Table 1**) (Dumbrava *et al.*, 2018). Here, the focus will be spotted on the two most frequently altered genes of this pathway.

Table 1. FDA approved drugs targeting the signaling of 18 cancer-related genes of the RTK-RAS pathway. Drugs that specifically target only the corresponding gene are shown in blue color. Drugs granted with a Breakthrough Therapy Designation but not yet approved by the FDA, are not included in this table.

Gene Signaling	Drugs
ABL1	Bosutinib, Brigatinib, Dasatinib, Ibrutinib, Imatinib, Niotinib, Pazopanib, Ponatinib, Regorafenib, Sunitinib, Tivozanib, Vandetanib
ALK	Alectinib, Brigatinib, Ceritinib, Crizotinib, Entrectinib, Gilteritinib, Lorlatinib, Sunitinib
BRAF	Binimetinib, Cobimetinib, Dabrafenib, Dasatinib, Encorafenib, Regorafenib, Sorafenib, Trametinib, Vemurafenib
EGFR	Afatinib, Brigatinib, Ceritinib, Cetuximab, Dacomitinib, Erlotinib, Gefitinib, Ibrutinib, Lapatinib, Lorlatinib, Mobocertinib, Necitumumab, Neratinib, Osimertinib, Panitumumab, Vandetanib.
ERBB2	Afatinib, Dacomitinib, Everolimus, Gefitinib, Ibrutinib, Lapatinib, Margetuximab, Metformin, Mobocertinib, Nelfinavir, Neratinib, Pertuzumab, Sirolimus, Temsirolimus, Trastuzumab, Trastuzumab Emtansine, Trastuzumab-ANNS, Trastuzumab/Hyaluronidase-oysk
FGFR1	Brigatinib, Dasatinib, Erdafitinib, Infigratinib, Lenvatinib, Nintedanib, Pazopanib, Ponatinib, Regorafenib, Sorafenib, Sunitinib, Tivozanib, Vandetanib
FGFR2	Brigatinib, Ceritinib, Erdafitinib, Infigratinib, Lenvatinib, Nintedanib, Pazopanib, Regorafenib, Sorafenib, Sunitinib, Vandetanib
FLT3	Brigatinib, Cabozatinib, Ceritinib, Fedratinib, Gilteritinib, Ibrutinib, Midostaurin, Nintedanib, Pexidartinib, Sorafenib, Sunitinib, Vandetanib
KIT	Axitinib, Cabozatinib, Dasatinib, Fedratinib, Imatinib, Infigratinib, Lenvatinib, Midostaurin, Nilotinib, Pazopanib, Pexidartinib, Ponatinib, Regorafenib, Sorafenib, Sunitinib, Tivozanib
KRAS	Binimetinib, Cobimetinib, Sotorasib, Trametinib
MET	Cabozatinib, Capmatinib, Crizotinib, Tepotinib, Tivozanib
NRAS	Binimetinib, Cobimetinib, Trametinib
NTRK1	Cabozatinib, Crizotinib, Entrectinib, Larotrectinib, Lorlatinib, Regorafenib, Sorafenib, Sunitinib
NTRK2	Cabozatinib, Entrectinib, Larotrectinib, Lorlatinib, Sorafenib, Sunitinib
PDGFRA	Axitinib, Dasatinib, Ibrutinib, Imatinib, Lenvatinib, Midostaurin, Nilotinib, Nintedanib, Olaratumab, Pazopanib, Ponatinib, Regorafenib, Sorafenib, Sunitinib, Tivozanib
PTPN11	Binimetinib, Cobimetinib, Trametinib
RET	Alectinib, Brigatinib, Cabozatinib, Ceritinib, Fedratinib, Ibrutinib, Lenvatinib, Pazopanib, Ponatinib, Pralsetinib, Regorafenib, Selpercatinib, Sorafenib, Sunitinib, Vandetanib
ROS1	Brigatinib, Cabozatinib, Ceritinib, Crizotinib, Entrectinib, Lorlatinib

After many years where *KRAS* gene was considered untargetable (Cox *et al.*, 2014; Keeton *et al.*, 2017; Liu *et al.*, 2019; Sheridan *et al.*, 2020), in 2021, a milestone in anti-cancer drug discovery was completed with the approval of the first KRAS-targeted therapy; sotorasib, an eclectic KRAS G12C inhibitor, was approved for adult NSCLC patients who had previously been treated with at least one systemic therapy (Blair *et al.*, 2021). Another selective *KRAS* G12C inhibitor, adagrasib, owing to its tolerability and clinical effectiveness, has granted a Breakthrough Therapy Designation by the FDA and its approval is expected to come soon (Riely *et al.*, 2021). With several more clinical trials underway (e.g. JDQ443 / NCT04699188, Mesenchymal Stromal Cells-derived Exosomes with KRAS G12D siRNA / NCT03608631 and Anti-KRAS G12V mTCR PBL / NCT03190941), a new era seems to be emerging for cancer treatment.

Over the last ten years, 3 selective $BRAF^{V600E}$ inhibitors have been approved from the U.S. Food and Drug Administration: vemurafenib (Flaherty *et al.*, 2011), dabrafenib (Ballantyne *et al.*, 2013) and encorafenib (Shirley *et al.*, 2018). These therapeutic agents, especially in combination with MEK inhibitors cobimetinib, trametinib and binimetinib respectively, have provided substantial progress-free survival (PFS) and overall survival (OS) benefit to melanoma, non-small cell lung cancer and thyroid cancer patients harboring this specific variant (Robert *et al.*, 2015; Garnock-Jones *et al.*, 2015; Dummer *et al.*, 2018; Odogwu *et al.*, 2018; Ascierto *et al.*, 2020; Salama *et al.*, 2020).

2.3. Lipid metabolism

Both lipid synthesis and catabolism are essential for the cell, given the broad range of cellular processes where these are involved in, including maintenance of membrane functionality, protein trafficking, immune cell responses, signaling, as well as coverage of metabolic demands such as energy production and storage (Zhou *et al.*, 2004; Groux-Degroote *et al.*, 2008; Wang *et al.*, 2010; Hishikawa *et al.*, 2014; Gyamfi *et al.*, 2019). It has been shown that cancer cells, in order to retain a high proliferative potential, they have to reset some aspects of their metabolism and this may happen through genetic alterations in genes regulating both processes (DeBerardinis *et al.*, 2008; Vander *et al.*, 2009; Hanahan *et al.*, 2011).

According to the present analysis, more than 40 % of patients of one in three cancer types / subtypes are characterized by driver mutations in genes involved in lipids metabolism. In Uterine Endometrioid Carcinoma, Astrocytoma, Oligodendroglioma and Oligoastrocytoma this proportion touched or exceeded 80 %, while, as with the two pathways discussed above, no primary Uveal Melanoma tumor of this dataset was affected

(**Figure 3**). At the gene level, *PIK3CA*, *PTEN* and *IDH1* were found genetically altered in 45.21 %, 27.56 % and 16.93 % of patients with a disorder in the related pathway respectively, occupying the first places in the mutagenicity ranking.

Over 95 % of *PIK3CA* somatic alterations in cancer are missense mutations with three substitutions comprising almost half of them: E545K, H1047R and E542K. All these substitutions exert a gain-of-function impact on the PI3K protein but through two different mechanisms. Amino acid residues E545 and E542 are located in the helical domain of p110alpha protein and their substitution by a lysine residue attenuates the inhibitory interaction between the catalytic subunit (p110alpha) and the nSH2 domain of the regulatory subunit (p85alpha) of PI3K protein, promoting the continuous activation of PI3K (Zhao *et al.*, 2008; Leontiadou *et al.*, 2018). A constitutive PI3K activation is also the result of the kinase domain variant H1047R, but this time, p110alpha abolishes its C-terminal tail's self-inhibitory capacity (Gkeka *et al.*, 2014). Both mechanisms lead to a strong activation of PI3K downstream effectors, such as AKT and P70S6K proteins, which mediate protein synthesis, cell growth, cell proliferation, angiogenesis, survival and thus contribute to tumorigenic transformation (Ikenoue *et al.*, 2005; Kang *et al.*, 2005; Bader *et al.*, 2006; Dogruluk *et al.*, 2015). At the same time, the hyper-activated PI3K/AKT signaling induces the expression of genes involved in fatty acids anabolism, a process that will generate building blocks essential for the new cells construction (Röhrig *et al.*, 2016; Snaebjornsson *et al.*, 2020).

PTEN alterations show greater variety than *PIK3CA* alterations, with missense mutations, frameshift indels and non-sense mutations representing 91 % of all carriers. The most prevalent mutations involve R130, R233 and T319 amino acid residues and are found in 17.5 %, 5 % and 4.74 % of all affected patients. In the majority of cases, arginine in position 130 is either substituted by a glutamine or a glycine residue, or, as in the cases of R233 and T319, the codon that is responsible for encoding it, is converted to a stop codon. Whereas truncating mutations such as R130*, R233* and T319* lead to an unstable, non-functional protein product (Georgescu *et al.*, 1999; Papa *et al.*, 2014; Rashmi *et al.*, 2014), R130Q and R130G variants generate stable proteins, which however lack their phosphatase activity (Papa *et al.*, 2014; Leslie *et al.*, 2014). Absence or non-functionality of the PTEN protein prevents PIP3 dephosphorylation, which then accumulates and recruits PI3K signaling effectors such as AKT proteins and PDK1 (Salmena *et al.*, 2008). Furthermore, it was recently shown that $PTEN^{R130Q}$ mutants tend to accumulate at the cell periphery where they form leading edges that increase tumor invasiveness and further activate PI3K/AKT signaling axis (Choi *et al.*, 2021).

A very impressive paradigm of a predominant mutational hotspot is offered by the *IDH1* gene. Of the 467 somatic driver mutations that were identified in an equal number of cancer patients, 466 involved an R132 replacement, with histidine being the most frequent substitute (388/466 cases), while cysteine, glycine, serine and leucine substitutions occurred in 50, 16, 11 and 1 patients respectively. These variants, which are mapped in the catalytic pocket of the enzyme, make isocitrate dehydrogenase-1 convert the normal final product of its catalytic activity, alpha-ketoglutarate (aKG), into R(−)-2-hydroxyglutarate (2HG), with a concomitant NADPH consumption (Kloosterhof *et al.*, 2011; Clark *et al.*, 2016). The following 2HG-mediated inhibition of aKG-dependent deoxygenases, such as TET2 and JMJD2A/C, is able to cause global gene expression changes which along with the redox stress arising from the declined NADPH levels, reflect the tumorigenic impact of these mutations (Dang *et al.*, 2010; Shim *et al.*, 2014; Parker *et al.*, 2015; Clark *et al.*, 2016). Likewise, even though the decreased levels of two lipogenesis components, NADPH and aKG (Yang *et al.*, 2012), would be expected to abrogate lipid synthesis, certain lipid precursors, such as glycerol-phosphates and glycerophosphocholine are present in elevated quantities, while others such as myo-inositol phosphate, are present in reduced levels compared to unaffected tumors, suggesting that cancer cells harboring IDH1 variants, alter their phospholipid expression profile, probably in a tumor-assisting manner (Reitman *et al.*, 2011; Esmaeili *et al.*, 2014; Zhou *et al.*, 2019).

As made clear from the above, signaling changes involving lipids metabolism regulators perturb a wide spectrum of cellular processes and contribute to cancer development. This observation has led to the development of therapies that mitigate these changes. Currently, there are 11 clinically available therapies that target the signaling of 4 out of the 24 lipid metabolism-related genes for which driver mutations in this dataset were found (**Table 2**) (Dumbrava *et al.*, 2018).

Table 2. FDA approved drugs targeting the signaling of 4 cancer-related genes of the lipid metabolism pathway. Drugs that specifically target only the corresponding gene are shown in blue color.

Gene Signaling	Drugs
IDH1	Ivositenib
PIK3CA	Alpelisib, Copanlisib, Duvelisib, Everolimus, Metformin, Midostaurin, Sirolimus, Temsirolimus
PIK3R1	Alpelisib, Copanlisib, Duvelisib, Everolimus, Idelalisib, Midostaurin, Sirolimus, Temsirolimus, Umbralisib
PTEN	Alpelisib, Copanlisib, Duvelisib, Everolimus, Metformin, Midostaurin, Sirolimus, Temsirolimus

As the results of this study suggest, when lipid metabolism is dysregulated on account of a genetic change, this alteration invariably affects one of its aforementioned PI3K/AKT signaling-mediating regulators. Hence, inhibition of PI3K/AKT signaling axis is the main goal of personalized medicine for the treatment of tumors bearing such alterations. When PI3K catalytic subunit inhibitors, both isoform-specific, such as alpelisib, and pan-isoform, such as copanlisib and duvelisib, as well as selective MTOR inhibitors, including everolimus and temsirolimus, are employed, they seem to offer a significant PFS – or even OS - benefit to patients dealing with a variety of blood or solid malignancies (Hudes *et al.*, 2007; Motzer *et al.*, 2008; Benjamin *et al.*, 2011; Markham *et al.*, 2017; Blair *et al.*, 2018; Markham *et al.*, 2019; Hasskarl *et al.*, 2018). In addition, ivosidenib, an inhibitor of all $IDH1^{R132}$ mutants, has been approved by the FDA for the treatment of acute myeloid leukemia (AML) patients harboring these variants (Dhillon *et al.*, 2018), while its effectiveness in other *IDH1*-deficient cancer types is tested in several ongoing clinical trials (e.g. advanced and metastatic cholangiocarcinoma / NCT02989857, chondrosarcoma / NCT04278781, cholangiocarcinoma – chondrosarcoma – glioma – other advanced solid tumors / NCT02073994, recurrent ependymoma – recurrent Ewing Sarcoma – recurrent hepatoblastoma / NCT04195555 etc).

2.4. The PI3K/AKT pathway

The impact PI3K/AKT signaling network has on diverse cellular functions is well established. Many of these, including cell growth and proliferation, survival, motility, cellular metabolism, immune system functions and angiogenesis are tightly intertwined with cancer development and progression and as a result a lot of research has been dedicated to unraveling the deregulation mechanisms of this pathway in cancer (Katso *et al.*, 2001; Engelman *et al.*, 2006; Courtney *et al.*, 2010; Fruman *et al.*, 2017).

The importance of PI3K/AKT axis deregulation for cancer development is reflected by the fact that in 26 out of the 36 cancer types of this dataset, more than 10 % of patients carried at least one driver mutation in genes involved in this pathway. Uterine malignancies showed the highest mutational rates with 93.3 % of Uterine Endometrioid Carcinoma patients being affected, while the corresponding proportion of the remaining uterine tumors analyzed also exceeded 60 %. On the other hand, less than 5 % of Serous Ovarian Cancer, Papillary Thyroid Cancer, Pheochromocytoma, Uveal Melanoma and Acute Myeloid Leukemia patients appeared to bear such changes (**Figure 3**). Among all PI3K/AKT-deregulated tumors examined, 53.75 % harbored driver mutations in *PIK3CA*,

32.76 % in *PTEN* and 12.93 % in *PIK3R1*, with the rest of the genes in this pathway being altered in less than 6 % of the tumors each. Interestingly, simultaneous genetic alterations in two or even all of the above mentioned top mutated genes, were present in 16.34 % of these patients. As driver mutations and therapeutic applications regarding *PIK3CA* and *PTEN* genes were discussed earlier in this report, now the *PIK3R1* gene will be discussed.

Somatic mutations in *PIK3R1* gene exhibit a highly scattered pattern. The absence of predominant hotspots is reflected by the mutational rates of the most prevalent genetic changes of this gene; 6.67 % for R348*, 5.67 % for X582_splice and 4 % for G376R. R348* is a truncating but gain-of-function mutation that exerts its tumorigenic impact in both a PI3K/AKT-dependent and a PI3K/AKT-independent manner. In addition to activating PI3K/AKT signaling, p85alpha mutants, by being localized into the nucleus, promote the activation of ERK and JNK kinases, thereby inhibiting FASL-mediated apoptosis and inducing cell survival, growth, proliferation and invasion (Seton-Rogers *et al.*, 2014; Cheung *et al.*, 2014). Even though X582_splice alteration has been previously identified and considered pathogenic (Moukarzel *et al.*, 2021), however its exact functional consequences have not been elucidated yet. Finally, the nSH2 domain-located G376R substitution, acts in the same way as the previously discussed E545 and E542 substitutions of p110alpha protein, thus attenuating the inhibitory interaction between the regulatory and the catalytic subunit of PI3K complex and enhancing the PI3K/AKT signaling (Sun *et al.*, 2010; Li *et al.*, 2021).

Overall, the PI3K/AKT signaling-promoting behavior of p85alpha mutants, places PIK3R1-targeting in the same therapeutic context as the other major effectors of this pathway, *PIK3CA* and *PTEN*, and as it is shown in **Table 2**, the available agents targeting these genes' signaling are in essence the same. In addition to PI3K and MTOR inhibitors, significant efforts are being made for developing AKT inhibitors (Song *et al.*, 2019; Martorana *et al.*, 2021). Most of the ongoing clinical trials test agents exerting a pan-AKT (AKT1, AKT2 and AKT3) inhibition. Of those, capivasertib and ipatasertib have demonstrated the most encouraging results and are now tested in several phase III trials (NCT03997123/ NCT04493853/ NCT04862663/ NCT04305496 and NCT03072238/ NCT03337724/ NCT04060862/ NCT04650581/ NCT04177108 respectively), mainly regarding breast and prostate cancer patients.

2.5. The ubiquitin pathway

Ubiquitination is a reversible modification that leads either to protein degradation or

to the regulation of protein-protein interactions. Many proteins' function is adjusted this way, making ubiquitination essential for the appropriate execution of an assortment of cellular events, namely inflammation, translation, endocytosis, DNA damage response, protein trafficking, differentiation and signal transduction (Pickart *et al.*, 2000; Deng *et al.*, 2000; Spence *et al.*, 2000; Hicke *et al.*, 2001; Katzmann *et al.*, 2002; Huen *et al.*, 2007; Mukhopadhyay *et al.*, 2007). Thus, deregulation of ubiquitin pathway can lead to cancer initiation and/or progression.

Mutations in genes encoding components or regulators of ubiquitination machinery are not uncommon in cancer samples. The present analysis demonstrates that such alterations are present in more than 10 % of patients in two out of three cancer types (24/36). The highest rates belonged to Uterine Carcinosarcoma, Mucinous Adenocarcinoma of the Colon and Rectum, Uterine Endometrioid Carcinoma and Breast Invasive Lobular Carcinoma, ranging from ~42 % to ~57 %. Contrariwise, Oligodendroglioma, Papillary Thyroid Cancer, Leiomyosarcoma, Uveal Melanoma and Pheochromocytoma exhibited the lowest rates with less than 3 % of the patients affected (**Figure 3**). At the gene level, the most frequently mutated genes, *FBXW7*, *EP300* and *CREBBP* were found mutated in 19.6 %, 10.72 % and 9.88 % of ubiquitination/deubiquitination-deficient tumors, while the mutational rate of 9 more genes ranged between ~5 % and ~9 % (in descending order of mutational frequency: *KMT2B*, *RNF43*, *VHL*, *MAP3K1*, *KMT2A*, *SPOP*, *BAP1*, *KEAP1* and *BRCA1*).

Three arginine residues of FBW7 protein - R465, R505 and R479 - represent 43.59 % of all FBW7 somatic driver mutations in primary tumor samples. All these positions are located into the WD40 domain. This domain serves as the substrate-binding site of the SCF complex (a type of E3 ligase), which is responsible for the ubiquitin-labeling and the subsequent proteasome-mediated degradation of its protein effectors (Yumimoto *et al.*, 2015; Yeh *et al.*, 2018). The superficial localization these residues have in the WD40 domain, makes their substitution capable of precluding the interaction potential of FBW7, probably by changing both hydrophobic and electrostatic interactions with its substrates, as well as by limiting the contact surface owing to the shorter substitutes' sidechains (mainly cysteine, histidine, glycine and glutamine) (Close *et al.*, 2019). Given that several FBW7 interactors act as regulators of cell growth, apoptosis and proliferation (Oberg *et al.*, 2001; Koepp *et al.*, 2001; Yada *et al.*, 2004; Wei *et al.*, 2005; Mao *et al.*, 2008; Inuzuka *et al.*, 2011), prevention of their degradation may result in tumorigenesis.

Even though *EP300* gene is usually altered by non-sense mutations, however, the

most recurrent changes belong to three other categories. The D1399N substitution is the most prevalent somatic mutation of this gene, accounting for 7.29 % of *EP300*-mutated primary tumors in the working dataset. Missense mutations in this position have been proved to change the conformation of the p300 protein histone acetyltransferase (HAT) domain, leading to abolishment of its autoacetylation activity, which is essential for appropriate function (Liu *et al.*, 2008; Delvecchio *et al.*, 2013). The subsequent inability of p300 to stimulate other tumor suppressors in the nucleus, such as RB1, BRCA1, p53 and AP-2alpha (Grossman *et al.*, 2000; Pao *et al.*, 2000; Chan *et al.*, 2001; Friedrichs *et al.*, 2005; Salloum *et al.*, 2017), paves the way for the predominance of its spatial distinct, cytoplasmic ubiquitin ligase activity, which targets p53 for degradation (Shi *et al.*, 2009). These changes, together with the reduced global levels of histone H3 acetylation (Attar *et al.*, 2017), contribute to the tumorigenic impact of this genetic alteration. The second and third most prevalent mutations of the *EP300* gene are the frameshift deletion M1470Cfs*26 and the splice site variant X1429_splice, and were each identified in 2.6 % of *EP300*-affected patients. However, their functional impact is not clear yet.

Missense mutations involving R1446 are the most recurrent somatic genetic changes in *CREBBP* gene, followed by frameshift indels involving I1084 and substitutions of D1435, accounting for 7.91 %, 5.65 % and 2.82 % of *CREBBP*-affected primary tumors respectively. Both R1446 and D1435 are located into the HAT domain, which is responsible for the catalytic activity of CBP transcriptional coactivator. Substitution of these amino acid residues, reduces the acetyl-CoA binding affinity of CBP, thereby impairing its acetyltransferase activity (Pasqualucci *et al.*, 2011; Peifer *et al.*, 2012; Merk *et al.*, 2018). Consequently, CBP can neither activate p53 tumor suppressor nor inactivate BCL6 proto-oncoprotein. Furthermore, as with its structurally and functionally related p300 protein, CBP also exhibits an E4 ubiquitin ligase activity targeting p53 for degradation in the cytoplasm (Shi *et al.*, 2009). These observations, in conjunction with the arised extended transcriptional changes, dictate a tumor promoting effect for these mutations (Pasqualucci *et al.*, 2011; Mondello *et al.*, 2020). The functional impact of frameshift deletion I1084Sfs*15, which is found in 4.52 % of *CREBBP*-altered samples thus far remains obscure.

Proteins involved in either the attachment or the removal of ubiquitin moieties are barely exploited in clinical practice (Deng *et al.*, 2020). An exception to this rule is provided by thalidomide analogues lenalidomide and pomalidomide, which have been approved for the treatment of various blood malignancies and exhibit their anti-tumor activity by changing the specificity of cereblon protein, the substrate recognition component of CRL4

E3 ubiquitin ligase complex (Lu *et al.*, 2014; Stewart *et al.*, 2014). Nonetheless, targeting of the proteolytic machine is primarily oriented to proteasome inhibition, with three inhibitors - bortezomib, carfilzomib and ixazomib - already approved for the treatment of multiple myeloma and mantle cell lymphoma (Fricker *et al.*, 2020), while other agents, such as CEP-18770 and NPI-0052 (marizomib) (Dick *et al.*, 2010) are currently being tested in clinical trials as potential treatment options for solid tumors (NCT00572637 and NCT03345095 respectively).

2.6. The WNT/b catenin pathway

The WNT/b catenin pathway is one of the best-studied signaling cascades in cancer development. This robust association is underscored by the pathway's implication in many cancer-related cellular functions such as cell proliferation, stem cell maintenance, differentiation, cell-cell adhesion, morphogenetic processes, migration, angiogenesis and immune evasion (Nelson *et al.*, 2004; Gattinoni *et al.*, 2009; Petersen *et al.*, 2009; Spranger *et al.*, 2015; Junge *et al.*, 2017).

Mutations of WNT/b catenin components are common in cancer patients. However, this analysis showed that this happens in a more limited number of cancers compared to the pathways discussed so far. Specifically, the proportion of patients with at least one driver mutation in this pathway exceeded 10 % in only 9/36 cancer types examined. Intestinal malignancies appeared in the forefront of the mutational landscape with at least 80 % of Colon Adenocarcinoma, Rectal Adenocarcinoma and Mucinous Adenocarcinoma of the Colon and Rectum patients harboring driver alterations. On the other hand, no such alterations were found in Uveal Melanoma or Leiomyosarcoma patients (**Figure 3**). Among WNT/b catenin effectors, the *APC*, *CTNNB1* and *RNF43* genes were found mutated in 48.59 %, 26.41 % and 14.94 % of the affected samples, respectively, while four other genes (in descending order of mutational frequency: *AMER1*, *AXIN1*, *TCF7L2* and *AXIN2*) exhibited a driver mutational rate between ~5 % and ~8 %.

The most frequent alteration type in *APC* gene is non-sense mutation, present in 40,6 % of patients. Conversion of arginine-encoding codons into stop codons predominantly takes place at positions 1450, 876 and 1114 of APC protein (8.51 %, 4.84 % and 4.64 % respectively). R1450 is located into the so called mutation cluster region (MCR) (Miyoshi *et al.*, 1992). Truncation of APC protein in this position leads to the loss of all axin- and most b catenin-binding sites, therefore abrogating the ability of APC to negatively regulate b catenin via the formation of the destruction complex AXIN-APC-CK1alpha-

GSK3beta (Fearnhead *et al.*, 2001; Azzopardi *et al.*, 2008; Pai *et al.*, 2017; Imperial *et al.*, 2018). Similar is the case regarding the two other truncations, as R1114* mutant also loses the axin- and most b catenin-binding sites, whereas R876* variant loses all these sites (Ficari *et al.*, 2000; Mihalatos *et al.*, 2003). In all three cases, the subsequent b catenin accumulation and nuclear entry permits the TCF/LEF1 transcriptional complex activation and thereby promotes cellular proliferation and tumorigenesis (Pai *et al.*, 2017).

Somatic mutations in *CTNNB1* gene are almost exclusively missense mutations. In particular, amino acid substitutions at six specific positions (32, 33, 34, 37, 41 and 45) represent more than 85 % of all affected patients in this dataset. Among them, S33, S37 and D32 replacements were proved the most prevalent, affecting 18.51 %, 17.44 % and 16.01 % of all cases. The protein region between D32 and S45 participates in the phosphorylation of b catenin from CK1alpha and GSK3beta (both components of its destruction complex), as well as in the interaction of phosphorylated b catenin with its E3 ligase substrate recognition component, FBW1 (Kitagawa *et al.*, 1999; Kikuchi *et al.*, 2003). Consequently, mutations in these protein sites exert similar signaling implications to the ones exerted by *APC*, as b catenin escapes proteasomal degradation and confers an increased proliferative potential to the cell (Morin *et al.*, 1997; Rubinfeld *et al.*, 1997; Liu *et al.*, 2002; Kikuchi *et al.*, 2003; Rebouissou *et al.*, 2016).

Almost seventy-three percent of *RNF43*-mutated primary tumors carry a frameshift deletion in this gene, while the second most frequent alteration type is a non-sense mutation (~17 %). By far the most common alteration of the RNF43 protein is G659Vfs*41, being present in approximately 60 % of all *RNF43*-mutated tumors. Interestingly, despite its recurrent presence in cancer samples, this frameshift deletion leaves protein function intact, with the relevant RNF43-mutant being able to exert its E3 ubiquitin ligase activity (Tu *et al.*, 2019); this results in the tagging of FZD family (frizzled transmembrane proteins) WNT receptors for proteasomal degradation and in the inactivation of WNT/b catenin signaling (Koo *et al.*, 2012; Hao *et al.*, 2012). The second and third most frequent somatic mutations of the RNF43 protein are R519* and R145*, affecting only 2.52 % and 1.89 % of all carriers. Both of them lead to a truncated protein with a WNT signaling-enhancing role, however the exact mechanism differs depending on the truncation position. The catalytic RING domain of RNF43 lies among P270 and I316 (Tu *et al.*, 2019; UniProt Consortium, 2021), and as such the R519* mutant retains its E3 ubiquitin ligase activity. Nonetheless, it simultaneously gains the ability to snare CK1alpha at the plasma membrane, thus assisting b catenin to escape degradation (Spit *et al.*, 2020). On the other hand, R145* variants lack this catalytic activity and are therefore expected to abort their FZD degradation and b

catenin destabilization role (Tu *et al.*, 2019).

Significant efforts have been made regarding WNT/b catenin network targeting, mainly for the development of small molecule stabilizers of the b catenin destruction complex components or destabilizers of the b catenin-TCF/LEF interaction, as well as antibodies and regulatory peptides that directly or indirectly affect - mostly inhibit - WNT or FZD proteins (Anastas *et al.*, 2013; Pai *et al.*, 2017; Jung *et al.*, 2020). Four such constructs have exhibited the most encouraging results and their efficacy is currently evaluated in phase II clinical trials of both solid and blood cancer patients. In this context, WNT974 (NCT02278133) – a porcupine inhibitor that precludes WNT secretion and activity, Foxy-5 (NCT03883802) – a WNT5a mimetic, PRI-724 (NCT01606579) – an antagonist of the b catenin coactivator CBP, and DKN-01 (NCT03395080) – a monoclonal antibody that neutralizes the activity of WNT/b catenin axis inhibitor DKK1, are now in the spotlight of WNT pathway targeting.

2.7. The Notch pathway

Notch is a cancer-related signaling pathway well known for its involvement in a variety of developmental processes, as well as cellular differentiation, proliferation, stem cell maintenance, angiogenesis, EMT, inflammation and apoptosis (Hori *et al.*, 2013; Bray *et al.*, 2016; Siebel *et al.*, 2017). Notch pathway compounds were found genetically changed at least to one in ten patients in fifteen out of thirty-six assessed cancer types. Uterine Carcinosarcoma exhibited the highest driver mutational rate (40.35 %), followed by Mucinous Adenocarcinoma of the Colon and Rectum (33.93 %) and Bladder Urothelial Carcinoma (29.02 %), while other gynecological and upper digestive tract malignancies were also highly affected. On the other side, no Pheochromocytoma patient and no more than 2 % of Leiomyosarcoma, Uveal Melanoma and Papillary Thyroid Cancer patients carried such alterations (**Figure 3**). At the gene level, the most frequently mutated components or regulators of Notch pathway appeared to be identical to the above discussed ubiquitination pathway's; *FBXW7*, *EP300* and *CREBBP* were found altered in 33.65 %, 18.41 % and 16.97 % of Notch-impaired tumors respectively, while somatic driver events in each of the *SPEN*, *NCOR1* and *NOTCH1* genes, were detected in ~12 % of cases.

Upon NOTCH receptors activation by their ligands (e.g. Delta-like protein 1, protein jagged-1 etc), the NOTCH-intracellular domain (NICD) is released and transferred into the nucleus where it acts as a transcription regulator. NICD is one of the FBW7 substrates

(Oberg *et al.*, 2001) and mutations within the FBW7 WD40 domain have been reported to impede this interplay, therefore leading to NICD accumulation and enhanced Notch signaling which may lead to tumorigenic outcomes (Pancewicz *et al.*, 2010; Yeh *et al.*, 2016; Close *et al.*, 2019). Furthermore, despite the previously reported p300 requirement for NICD transcriptional activity manifestation (Oswald *et al.*, 2001; Wallberg *et al. 2002*), it was recently demonstrated that loss of function mutations in either the *EP300* or *CREBBP* gene can also activate Notch axis due to the subsequent low histone acetylation levels of *FBXW7* promoter (Huang *et al.*, 2021), suggesting the existence of additional transcriptional NICD co-activators.

So far, no targeted therapies for Notch signaling regulation have entered clinical practice. However, remarkable efforts have been made to overcome the obstacles Notch pathway places in this route, given the highly context-specific behavior of this signaling axis in cancer (Majumder *et al.*, 2021). To this end, diverse strategies have been utilized, targeting NOTCH biosynthesis enzymes, receptor-ligand interplay, NOTCH cleavage-performing effectors or NICD-containing transcriptional complexes assemblage (Pannuti *et al.*, 2010; Takebe *et al.*, 2014; Majumder *et al.*, 2021). The most encouraging results come from the development of inhibitors against gamma-secretase - which is responsible for the final NICD-releasing NOTCH cleavage – and Delta-like protein 3, a NOTCH ligand, with some of the relevant cancer-related clinical trials being in advanced stages. Specifically, the efficacy of gamma-secretase inhibitors (GSIs) nirogacestat/PF-03084014 (NCT03785964) and MK0752 (NCT00756717) is currently evaluated in phase III trials in adults with desmoid tumors and early stage breast cancer patients in combination with tamoxifen respectively, while the tesirine conjugated anti-Delta-like protein 3 mAb rovalpituzumab (Rova-T) (NCT03061812) is also tested in a phase III clinical trial in small-cell lung cancer (SCLC) patients with disease progression following platinum-based chemotherapy and overexpressing Delta-like protein 3.

2.8. The cell cycle pathway

The cell cycle is a set of fine-tuned, strictly inspected processes which mediate between the end of two consecutive cell divisions and are responsible for the apt preparation of the cell toward a complete, equal and accurate cell material distribution between daughter cells. Consequently, functional disruption of key cell cycle mediators can provoke loss of proliferation control, as well as a disturbance of the genomic integrity, both of which constitute fundamental features of cancer (Malumbres *et al.*, 2009; Matthews *et al.*, 2021).

Driver somatic mutations of crucial cell cycle components are present in more than 10 % of patients in 25 % of cancer types studied. For some malignancies the occurrence rate exceeds even 20 %, with Bladder Urothelial Carcinoma, Head and Neck Squamous Cell Carcinoma and Lung Squamous Cell Carcinoma patients being affected in 30.24 %, 22.52 % and 20.04 % of cases respectively; at the same time no such alterations were found in Renal Clear Cell Carcinoma, Acute Myeloid Leukemia, Uveal Melanoma or Pheochromocytoma patients (**Figure 3**). Indisputably, *RB1* and the previously discussed *CDKN2A* are the most commonly changed cell cycle genes, as mutations of them were identified in 44.43 % and 41.11 % of all cell cycle-perturbed primary tumors respectively; despite ranking third in the relative ranking of mutational frequency, the *CDKN1A* gene was found mutated in 6.76 % of assessed tumors.

Somatic mutations were highly dispersed along *RB1* gene, with 203 of the 928 amino acid-encoding codons found to be implicated in a driver event in this dataset. Even though more than 40 % of these genomic changes were nonsense mutations, the most recurrent is the splice site mutation X405_splice, which nevertheless accounts for only ~3 % of *RB1*-mutated tumors. Among the nonsense alterations, R320* and R552* were the most prevalent, being present in 2.09 % of cases each. All three of these alterations, constitute inactivating mutations that impair both RB1 functional domains (A- and B-boxes) (Cowell *et al.*, 1994; Richter *et al.*, 2003; Ayari *et al.*, 2014; Yu *et al.*, 2018). Such alterations incapacitate one of the cell cycle restriction points, as a non-functional or absent RB1 protein permits E2F-mediated G1/S transition, thereby enhancing the cell's proliferative potential and contributing to tumorigenesis or tumor progression (Trimarchi *et al.*, 2002; Burkhart *et al.*, 2008).

Somatic driver genomic alterations in *CDKN1A* gene are extremely rare, as only 51 out of 10,066 (~0.5 %) assessed primary tumor samples appeared to be affected, with the majority detected in Bladder Urothelial Carcinoma (39/51) and Hepatocellular Carcinoma (7/51) patients. Frameshift indels and nonsense mutations represent the lion's share of *CDKN1A* gene alterations. Q10* and M38Nfs*10 are the most recurrent among them, accounting for 7.84 % and 5.88 % of p21-deficient tumors. Although experimentally uncharacterized, both mutations are expected to abrogate p21 functionality due to loss of most functional domains. Such aberrations are likely to assist tumor progression, as deficient p21 is unable to prevent cell cycle progression and DNA synthesis through cyclin-CDK complexes or PCNA inhibition (Xiong *et al.*, 1993; Waga *et al.*, 1994; Jackson *et al.*, 2002; Poole *et al.*, 2004; Forster *et al.*, 2008), while in the case of M38Nfs*10 frameshift

insertion, the intact 33 NH2-terminal amino acid residues, where procaspase-3 binding domain is located, might further facilitate tumor progression due to sustenance of p21 anti-apoptotic activity (Suzuki *et al.*, 1999; Yu *et al.*, 2005).

Thus far, therapeutic targeting of the cell-cycle is largely based on CDK4/6 inhibition (Suski *et al.*, 2021). Three such inhibitors, palbociclib, ribociclib and abemaciclib have already received FDA approval, either as monotherapy or in combination with hormone therapies, for the treatment of advanced HR+/HER2- breast cancer (Dhillon *et al.*, 2015; Syed *et al.*, 2017; Kim *et al.*, 2017). Recently, another CDK4/6 inhibitor, trilaciclib, was also approved for the prevention of chemotherapy-induced myelosuppression in small cell lung cancer (SCLC) patients (Dhillon *et al.*, 2021). In parallel, many more such inhibitors are currently being tested in clinical trials for their potential to confer a similar or even improved anti-tumor activity as the already available ones, but accompanied by less toxicity. Of these, dalpiciclib (SHR6390) is the only compound currently tested in phase III trials (NCT03966898 and NCT03927456), while many pan-CDK inhibitors are evaluated in earlier phases of clinical development (Dumbrava *et al.*, 2018). Apart from CDKs, several inhibitors of other cell-cycle components such as CHK1, PLKs and Aurora proteins have also entered clinical testing (Otto *et al.*, 2017).

2.9. The HDR pathway

Homology-directed DNA repair (HDR) is one of the two major mechanisms that are responsible for double strand breaks (DSBs) repair, and in fact the most precise one (Karran *et al.*, 2000; Chen *et al.*, 2018). Given the detrimental impact DSBs have on genomic stability (van Gent *et al.*, 2001), malfunction of HDR pathway could facilitate malignant transformation (Khanna *et al.*, 2001). The present analysis showed that somatic driver mutations in HDR pathway components are not rare events in cancer. In particular, 23.71 % of Uterine Endometrioid Carcinoma patients harbored such mutations, while the relative fraction of five gastrointestinal cancer types – Mucinous Adenocarcinoma of the Colon and Rectum, Stomach Adenocarcinoma, Diffuse Type Stomach Adenocarcinoma, Tubular Stomach Adenocarcinoma and Colon Adenocarcinoma – was 21.43 %, 17.39 %, 13.89 %, 12.66 % and 10.95 % respectively. In contrast, less than 1 % of Papillary Thyroid Cancer and Oligodendroglioma patients and no Oligoastrocytoma patients carried such alterations (**Figure 3**). Among HDR compounds, *BRCA2*, *BRCA1* and *TP53BP1* were the most frequently affected genes, being genetically altered in 25.17 %, 14.73 % and 13.01 % of HDR-impaired patients, respectively; the relative mutational rate of 8 additional genes (in descending order of mutational frequency: *BRIP1*, *RAD51B*, *NBN*, *RAD50*, *BLM*, *SLX4*,

BARD1 and *PALB2*) ranged between ~5 and ~9 %.

Almost 41 % of somatic *BRCA2* gene alterations are truncating mutations, most of which result in loss of multiple BRCA2 functional domains. However, the most recurrent BRCA2 protein changes are the frameshift insertion N1784Kfs*3, the frameshift deletion K1691Nfs*15 and the missense mutation R2842C, which were detected in 3.4 %, 2.72 % and 2.04 % of all *BRCA2*-mutated primary tumor samples in this cohort, respectively. The R2842 substitution by a cysteine residue has been demonstrated to mitigate HDR efficiency (Caburet *et al.*, 2020). Although N1784Kfs*3 and K1691Nfs*15 have been previously identified (Sun *et al.*, 2020), however their exact functional consequences remain unresolved. Nevertheless, the final shortened variants lack crucial BRCA2 C-terminal domains, such as some of the RAD51-binding sites, the DNA-binding domain and their nuclear localization sequences (NLSs) (Lee *et al.*, 2014; Chen *et al.*, 2018), likely dictating a loss of function effect. A dysfunctional BRCA2 protein is incapable of efficiently recruiting RAD51 at DSB sites, making RAD51 nucleoprotein filament formation difficult and therefore hindering homology-directed invasion of damaged DNA to the intact sister chromatid (Moynahan *et al.*, 2001; Liu *et al.*, 2010). The subsequent HDR deficiency makes cells to rely on other, error-prone DSB repair mechanisms (Tutt *et al.*, 2001), thereby contributing to genome instability, one of the cancer hallmarks.

As for *BRCA2*, nonsense is also the predominant mutation type of *BRCA1* gene (~41 %). At the protein level, the most common changes appeared to be the frameshift insertion E111Gfs*3 and the nonsense mutations E720* and E572*, which account the first for 3.49 % and each of the last two for 2.33 % of all *BRCA1*-affected tumors. Despite the previous identification of all three variants (Rebbeck *et al.*, 2018), their functional consequences have not been experimentally validated yet. However, the truncation sites of these mutants dictate the loss of coiled-coil domain, RAD50- and RAD51-binding domains and the two BRCT domains, while E572* and E111Gfs*3 variants are additionally expected to lack one or both BRCA1 nuclear localization sequences respectively (Scully *et al.*, 1997; Zhong *et al.*, 1999; Clark *et al.*, 2012; Christou *et al.*, 2013). Such variants are most likely unable to both prevent 53BP1-mediated inhibition of the initial HDR step, viz., DNA end resection by MRE11-RAD50-NBS1 (MRN) complex (Bunting *et al.*, 2010), and to recruit and stabilize the RAD51 protein onto the DSB sites (Christou *et al.*, 2013), thus resulting in HDR deficiency.

Nonsense mutations and frameshift deletions account for ~80 % of *TP53BP1* gene's driver somatic alterations in cancer. The corresponding most repetitive protein changes are

E737* (6.58 %), N1017Mfs*20 (6.58 %) and E711Nfs*12 (3.95 %). All three constitute truncating mutations leading to loss of both the NLS and the four C-terminal functional domains of 53BP1 protein. It was recently demonstrated that such changes result in significant retardation of DSB repair, suggesting impairment of the 53BP1-mediated non-homologous end joining (NHEJ) repair mechanism (Zhang *et al.*, 2021). Although 53BP1 can act as an HDR's negative regulator (Bunting *et al.*, 2010; Panier *et al.*, 2014), the predominant role of NHEJ on DSB repair, primarily during the G1 phase, when the HDR is inactive (Mao *et al.*, 2008), renders 53BP1 deficiency a genome stability-threatening situation.

Targeted therapy of HDR-deficient tumors is highly based on synthetic lethality concept (Yap *et al.*, 2011; Furgason *et al.*, 2013; Brown *et al.*, 2017), wherein mutations in different genes can lead to cell death when co-occurring. In this context, *BRCA1*/*2*-deficient tumors, which lack the ability of accurately repairing DSBs, can be treated with single strand break (SSB) repair inhibitors, such as inhibitors of the base excision repair-mediators PARP1/2 (Helleday *et al.*, 2008). Four such agents, olaparib, rucaparib, niraparib and talazoparib, are clinically available for the treatment of pretreated advanced breast, ovarian and fallopian tube or primary peritoneal cancer patients harboring BRCA1/2 mutations (this restriction does not apply for niraparib), either germline or/and somatic (Deeks *et al.*, 2015; Syed *et al.*, 2017; Scott *et al.*, 2017; Hoy *et al.*, 2018; Arora *et al.*, 2021). Several more PARPis are in clinical development with three of them, fluzoparib (NCT03863860), pamiparib (NCT03519230) and veliparib (NCT02470585, NCT02163694 and NCT02152982), currently being tested in phase III trials. In addition to BRCA1/2m – PARPis couple, increasing evidence demonstrates that synthetic lethality also occurs after ATR inhibition in HDR-deficient tumors carrying inactivated ATM (Kwok *et al.*, 2016; Menolfi *et al.*, 2020; Topatana *et al.*, 2020), with active ongoing phase II clinical trials testing two such agents: berzosertib (NCT02567409, NCT03517969 and NCT02595892) and ceralasertib (NCT03330847, NCT03328273, NCT03787680 and NCT02937818) in a variety of human cancers.

2.10. The splicing pathway

Splicing is the process of exon joining following intron exclusion that happens during the conversion of pre-mRNA to its mature translatable form. Under certain circumstances, an aberrant splicing procedure can prove oncogenic (David *et al.*, 2010; Dvinge *et al.*, 2016; Climente-González *et al.*, 2017; Rahman *et al.*, 2020). This study revealed that somatic driver events involving splicing machinery components are not uncommon in

malignancies. Thus, cancers from different anatomical regions, namely, Uveal Melanoma, Oligodendroglioma, Uterine Endometrioid Carcinoma, Lung Adenocarcinoma, Mucinous Adenocarcinoma of the Colon and Rectum and Bladder Urothelial Carcinoma exhibited an occurrence rate of 23.75 %, 18.52 %, 13.92 %, 10.95 %, 10.71 % and 10.24 % respectively. In contrast, less than 1 % of Glioblastoma Multiforme and Pheochromocytoma patients and no Papillary Thyroid Cancer or Leiomyosarcoma patient harbored such genetic changes (**Figure 3**). At the gene level, *SPEN*, *CDK12* and *FUBP1* appeared as the most commonly altered genes, being involved in 20.82 %, 19.89 % and 17.84 % of splicing-impaired tumors, whereas the corresponding proportion of four more genes fluctuated between ~8 % and ~14 % (in descending order of mutational frequency: *RBM10*, *SF3B1*, *DDX3X* and *U2AF1*).

The *SPEN* gene, which encodes for SMRT/HDAC1-associated repressor protein (SHARP or SPEN), is mostly affected by nonsense mutations and frameshift deletions (~81 % of driver events). These changes display a dotted appearance along the gene, something clearly dictated by the occurrence rate of the most recurrent protein alterations, I1052Sfs*40, A2251Qfs*102 and P2495Lfs*4 (2.68 % each). None of them has been functionally characterized so far. However, the undetectable SPEN protein levels accompanying an insertion/truncation in position 1184 (Légaré *et al.*, 2015), dictate a loss of function in I1052Sfs*40 variant as well. Furthermore, all three variants lack their C-terminal SPOC domain (UniProt Consortium, 2021), which is essential for SPEN transcriptional corepressor activity, as it is utilized for its interaction with SMRT/NCoR (Ariyoshi *et al.*, 2003). SPEN-deficient cells are incapable of hindering ERalpha oncogenic effects, thus facilitating tumor formation (Légaré *et al.*, 2015). However, the mechanism via which abolishment of SPEN splicing-related activity – likely a link between RNA splicing and mRNA nuclear export machines (Hiriart *et al.*, 2005) – may contribute to tumorigenesis, remains unclarified.

Nearly 80 % of patients affected by a somatic driver mutation in *CDK12* gene harbor a fusion, a frameshift deletion or a nonsense mutation. Nevertheless, R890H substitution is the most recurrent variation (3.74 %), followed by the frameshift deletion P683Qfs*70 and the CDK12-IKZF3 fusion (2.8 % each). The operational consequences of these particular alterations have not been experimentally delineated yet. The R890 is located into the kinase domain (aa residues 737 - 1020) (Bartkowiak *et al.*, 2015). Amino acid substitutions very close to this position have been demonstrated to either prevent CDK12 interaction with its protein partner cyclin K or considerably decrease CDK12's kinase activity (Ekumi *et al.*, 2015). In addition, P683Qfs*70 truncation makes the corresponding variant to lose

almost its entire kinase domain. The inability of aberrant CDK12 to phosphorylate the C-terminal domain of RNA polymerase II, makes it incapable of exerting its transcription-activating role on its several HDR pathway target genes as well as other DNA damage response (DDR) components (Blazek *et al.*, 2011; Ekumi *et al.*, 2015; Krajewska *et al.*, 2019). Furthermore, CDK12-deficiency may promote proximal alternative last exon (ALE) splicing of certain DDR genes, such as *ATM*, thus limiting the abundance of their full-length protein product (Tien *et al.*, 2017), and in this way impeding DNA repair processes and further contributing to genome instability and cancer development.

The *FUBP1* gene, encoding for far upstream element-binding protein 1 (FBP), displays a significant variety of mutation types, with the most frequent one, frameshift deletions, detected in 37.5 % of all carriers. The frameshift deletion S11Lfs*43 is the most frequently repeated somatic alteration, being present in 9.38 % of patients, followed by the R430C substitution (8.33 %) – the only missense mutation identified in 96 FBP-affected patients – and frameshift indels involving I301 (5.20 %). All these changes have been identified by massive sequencing approaches, but not yet characterized (Cancer Genome Atlas Network, 2012; Schneeweiss *et al.*, 2018; Yaeger *et al.*, 2018). Variants harboring either S11Lfs*43 or I301-involving out-of-frame mutations, namely deletions I301Yfs*22 and I301Nfs*22 as well as insertion I301Nfs*4, lose more than 90 % or at least half of their protein body respectively, which suggests a loss of function effect. Although known as a positive regulator of MYC proto-oncogene (Duncan *et al.*, 1994), it is the previously reported FBP participation in a MYC-repressing complex with FIR (Hsiao *et al.*, 2010) that is consistent with a tumor-promoting scenario. Additionally, it has been demonstrated that FBP loss hampers proper MDM2 splicing, giving rise to the tumorigenesis-accelerating *MDM2-ALT1* splice variant (Jacob *et al.*, 2014).

Therapeutic targeting of splicing pathway is still far from entering the clinic. However, strategies have been developed toward this direction. These include three main approaches: (i) spliceosome assembly disruption through the inhibition of core spliceosome components (e.g. SF3B1) or their regulators (e.g. SRPKs and CLKs) using small molecules, (ii) control of the utilized splicing regulatory elements (exonic and intronic splicing enhancers or silencers) or inhibition of cancer-related aberrantly expressed splicing factors by oligonucleotides and (iii) targeting of abnormal splice isoforms (Lee *et al.*, 2016; Urbanski *et al.*, 2018; Zhang *et al.*, 2021). Currently, the first strategy has come to the fore, being the only approach to include compounds under clinical evaluation. Thus, two SF3B-complex (a component of U2 snRNP) inhibitors, E7107 (NCT00459823 and NCT00499499) and H3B-8800 (NCT02841540) are now tested in phase I trials for their

safety and anti-tumor activity against solid and hematological malignancies, respectively.

3. DISCUSSION AND CONCLUSIVE REMARKS

In this study, the most popular cancer-related signaling pathways were sorted by their somatic driver mutational rate. By focusing on the first ten pathways in terms of mutational frequency, the cancer types where these cascades are most frequently found disturbed were outlined, the signaling consequences of the most repeatedly identified alterations/mutations in key members of these pathways were described and their therapeutic exploitation was discussed, while in several cases literature gaps were addressed, which, if covered, would undoubtedly upgrade cancer treatment opportunities.

In the main body of this article, it became clear that there is a remarkable diversity of mechanisms by which somatic mutations can drive cell signaling perturbance in cancer. This complexity becomes even wider if considering the simultaneous deregulation of multiple signaling cascades in many tumor types. For example, somatic driver mutations in key regulators of RTK-RAS, lipid metabolism and WNT/b catenin pathways, co-occurred in nearly 40 % of Mucinous Adenocarcinoma of the Colon and Rectum patients in the dataset examined. Furthermore, mutations in different regions of a single gene, may deregulate distinct signal transduction networks. Such a paradigm is offered by the R348* mutation of PIK3R1 which, in contrast to other protein region alterations, whose functional consequence is usually limited to PI3K/AKT axis activation, additionally enhances MAPK signaling, making cells sensitive to MEK and JNK inhibition (Cheung *et al.*, 2014). These insights underscore the importance of patient mutational profiling for therapeutic decision-making, as well as the value of filling knowledge gaps regarding the functional characterization of cancer-related variants.

Several recurrent cancer-related variants remain uncharacterized. These involve components of crucial signaling pathways, including PI3K/AKT, ubiquitination, cell cycle and splicing. *CREBBP* and *FUBP1* genes offer some of the most interesting cases. The cellular compartment-dependent roles of CBP either as a histone acetyltransferase or as an E4 ubiquitin ligase and their opposing impact on p53 activation (Shi *et al.*, 2009), make clarification of I1084-involving frameshift effects a very interesting task with potential therapeutic value. In regard to *FUBP1* gene, S11Lfs*43, R430C and the three identified I301-involving frameshift indels, together account for more than one fifth of *FUBP1*-affected tumors. The recent inclusion of *FUBP1* in the “long-tail driver” category of the less-frequently mutated genes, due to its alternative splicing regulatory role on several oncogenes and tumor suppressors (Elman *et al.*, 2019), renders the unraveling of such mutational effects, a highly attractive concept.

New horizons in cancer treatment can also arise from more adequately understood cancer contributors. Thus far, targeting of the ubiquitin-proteasome system (UPS) is primarily based on proteasome inhibition. However, the broad use of this strategy is limited by problems caused by the accumulated ubiquitin-labeled proteins (Deng *et al.*, 2020). Therefore, targeting earlier steps of the process might surmount this hurdle. The tumor-suppressor *FBXW7*, which is mutated in 1/5 of ubiquitination-deficient tumors, is an appealing target, given its role in the destabilization of many proto-oncoproteins (Sailo *et al.*, 2019). In addition, regarding the well-studied and clinically exploitable synthetic lethality concept, exploration of new DDR member couples apart from BRCA1/2-PARPs, may also prove valuable. Such an opportunity may be provided by *TP53BP1*, one of the most frequently mutated members of the major DNA DSB repair pathways (HDR and NHEJ), the driver mutations of which are mutually exclusive with those of *BRCA1*/*2* in 80 % of cases, thus providing an opportunity for the extension of the synthetic lethality concept to HDR-proficient tumors. The previously revealed synthetic lethal interaction between 53BP1 and DNA polymerase theta, the key mediator of another DSB repair mechanism called theta mediated end joining (TMEJ) (Wyatt *et al.*, 2016; Feng *et al.*, 2019), is in line with this notion.

Although focused on just one of the –omics areas, this work can still provide a useful overview considering the current knowledge on signaling pathway dysregulation in cancer. Understanding the impact molecular events like the above have on cell signaling would provide a better exploitation of the already existing targeted therapies, while it would also facilitate the enrichment of the current therapeutic arsenal with agents targeting frequently affected but still undruggable pathways such as splicing pathway, thus opening up new opportunities for more effective combinatorial therapies.

4. BIBLIOGRAPHY

1. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians, 71(3), 209–249.
2. Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. Nature reviews. Genetics, 17(6), 333–351.
3. Carrasco-Ramiro, F., Peiró-Pastor, R., & Aguado, B. (2017). Human genomics projects and precision medicine. Gene therapy, 24(9), 551–561.
4. Imai, K., & Takaoka, A. (2006). Comparing antibody and small-molecule therapies for cancer. Nature reviews. Cancer, 6(9), 714–727.
5. Sharma, P., & Allison, J. P. (2015). Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell, 161(2), 205–214.
6. O'Connor M. J. (2015). Targeting the DNA Damage Response in Cancer. Molecular cell, 60(4), 547–560.
7. Parui A.L., Bose K. (2019) Cancer Biology and Its Treatment Modalities: A Brief Historical

Perspective. In: Bose K., Chaudhari P. (eds) Unravelling Cancer Signaling Pathways: A Multidisciplinary Approach. Springer, Singapore.
8. Sever, R., & Brugge, J. S. (2015). Signal transduction in cancer. Cold Spring Harbor perspectives in medicine, 5(4), a006098.
9. Okugawa, Y., Grady, W. M., & Goel, A. (2015). Epigenetic Alterations in Colorectal Cancer: Emerging Biomarkers. Gastroenterology, 149(5), 1204–1225.e12.
10. Martincorena, I., & Campbell, P. J. (2015). Somatic mutation in cancer and normal cells. Science (New York, N.Y.), 349(6255), 1483–1489.
11. Baylin, S. B., & Jones, P. A. (2016). Epigenetic Determinants of Cancer. Cold Spring Harbor perspectives in biology, 8(9), a019505.
12. Feinberg, A. P., Koldobskiy, M. A., & Göndör, A. (2016). Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. Nature reviews. Genetics, 17(5), 284–299.
13. Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 24.2, Proto-Oncogenes and Tumor-Suppressor Genes
14. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. Cell, 144(5), 646–674.
15. Sanchez-Vega, F., Mina, M., Armenia, J., Chatila, W. K., Luna, A., La, K. C., Dimitriadoy, S., Liu, D. L., Kantheti, H. S., Saghafinia, S., Chakravarty, D., Daian, F., Gao, Q., Bailey, M. H., Liang, W. W., Foltz, S. M., Shmulevich, I., Ding, L., Heins, Z., Ochoa, A., … Schultz, N. (2018). Oncogenic Signaling Pathways in The Cancer Genome Atlas. Cell, 173(2), 321–337.
16. Vogelstein, B., & Kinzler, K. W. (2004). Cancer genes and the pathways they control. Nature medicine, 10(8), 789–799.
17. Dempke, W., Fenchel, K., Uciechowski, P., & Chevassut, T. (2017). Targeting Developmental Pathways: The Achilles Heel of Cancer?. Oncology, 93(4), 213–223.
18. Pon, J. R., & Marra, M. A. (2015). Driver and passenger mutations in cancer. Annual review of pathology, 10, 25–50.
19. Johnson, A., Zeng, J., Bailey, A. M., Holla, V., Litzenburger, B., Lara-Guerra, H., Mills, G. B., Mendelsohn, J., Shaw, K. R., & Meric-Bernstam, F. (2015). The right drugs at the right time for the right patient: the MD Anderson precision oncology decision support platform. Drug discovery today, 20(12), 1433–1438.
20. Saltz, L. B., Meropol, N. J., Loehrer, P. J., Sr, Needle, M. N., Kopit, J., & Mayer, R. J. (2004). Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 22(7), 1201–1208.
21. Shaw, A. T., Kim, D. W., Nakagawa, K., Seto, T., Crinó, L., Ahn, M. J., De Pas, T., Besse, B., Solomon, B. J., Blackhall, F., Wu, Y. L., Thomas, M., O'Byrne, K. J., Moro-Sibilot, D., Camidge, D. R., Mok, T., Hirsh, V., Riely, G. J., Iyer, S., Tassell, V., … Jänne, P. A. (2013). Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. The New England journal of medicine, 368(25), 2385–2394.
22. Zhan, T., Rindtorff, N., Betge, J., Ebert, M. P., & Boutros, M. (2019). CRISPR/Cas9 for cancer research and therapy. Seminars in cancer biology, 55, 106–119.
23. Khalaf, K., Janowicz, K., Dyszkiewicz-Konwińska, M., Hutchings, G., Dompe, C., Moncrieff, L., Jankowski, M., Machnik, M., Oleksiewicz, U., Kocherova, I., Petitte, J., Mozdziak, P., Shibli, J. A., Iżycki, D., Józkowiak, M., Piotrowska-Kempisty, H., Skowroński, M. T., Antosik, P., & Kempisty, B. (2020). CRISPR/Cas9 in Cancer Immunotherapy: Animal Models and Human Clinical Trials. Genes, 11(8), 921.
24. Sanchez-Vega, F., Mina, M., Armenia, J., Chatila, W. K., Luna, A., La, K. C., Dimitriadoy, S., Liu, D. L., Kantheti, H. S., Saghafinia, S., Chakravarty, D., Daian, F., Gao, Q., Bailey, M. H., Liang, W. W., Foltz, S. M., Shmulevich, I., Ding, L., Heins, Z., Ochoa, A., … Schultz, N. (2018). Oncogenic Signaling Pathways in The Cancer Genome Atlas. Cell, 173(2), 321–337.
25. Knijnenburg, T. A., Wang, L., Zimmermann, M. T., Chambwe, N., Gao, G. F., Cherniack, A. D., Fan, H., Shen, H., Way, G. P., Greene, C. S., Liu, Y., Akbani, R., Feng, B., Donehower, L. A., Miller, C., Shen, Y., Karimi, M., Chen, H., Kim, P., Jia, P., … Wang, C. (2018). Genomic and Molecular Landscape of DNA Damage Repair Deficiency across The Cancer Genome Atlas. Cell reports, 23(1), 239–254.
26. Seiler, M., Peng, S., Agrawal, A. A., Palacino, J., Teng, T., Zhu, P., Smith, P. G., Cancer

Genome Atlas Research Network, Buonamici, S., & Yu, L. (2018). Somatic Mutational Landscape of Splicing Factor Genes and Their Functional Consequences across 33 Cancer Types. Cell reports, 23(1), 282–296.
27. Ge, Z., Leighton, J. S., Wang, Y., Peng, X., Chen, Z., Chen, H., Sun, Y., Yao, F., Li, J., Zhang, H., Liu, J., Shriver, C. D., Hu, H., Cancer Genome Atlas Research Network, Piwnica-Worms, H., Ma, L., & Liang, H. (2018). Integrated Genomic Analysis of the Ubiquitin Pathway across Cancer Types. Cell reports, 23(1), 213–226.
28. Peng, X., Chen, Z., Farshidfar, F., Xu, X., Lorenzi, P. L., Wang, Y., Cheng, F., Tan, L., Mojumdar, K., Du, D., Ge, Z., Li, J., Thomas, G. V., Birsoy, K., Liu, L., Zhang, H., Zhao, Z., Marchand, C., Weinstein, J. N., Cancer Genome Atlas Research Network, … Liang, H. (2018). Molecular Characterization and Clinical Relevance of Metabolic Expression Subtypes in Human Cancers. Cell reports, 23(1), 255–269.
29. Thorsson, V., Gibbs, D. L., Brown, S. D., Wolf, D., Bortone, D. S., Ou Yang, T. H., Porta-Pardo, E., Gao, G. F., Plaisier, C. L., Eddy, J. A., Ziv, E., Culhane, A. C., Paull, E. O., Sivakumar, I., Gentles, A. J., Malhotra, R., Farshidfar, F., Colaprico, A., Parker, J. S., Mose, L. E., … Shmulevich, I. (2018). The Immune Landscape of Cancer. Immunity, 48(4), 812–830.
30. Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O., Aksoy, B. A., Jacobsen, A., Byrne, C. J., Heuer, M. L., Larsson, E., Antipin, Y., Reva, B., Goldberg, A. P., Sander, C., & Schultz, N. (2012). The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer discovery, 2(5), 401–404.
31. Levine, A. J., & Oren, M. (2009). The first 30 years of p53: growing ever more complex. Nature reviews. Cancer, 9(10), 749–758.
32. Aubrey, B. J., Strasser, A., & Kelly, G. L. (2016). Tumor-Suppressor Functions of the TP53 Pathway. Cold Spring Harbor perspectives in medicine, 6(5), a026062.
33. Oren M. (2003). Decision making by p53: life, death and cancer. Cell death and differentiation, 10(4), 431–442.
34. Harris, S. L., & Levine, A. J. (2005). The p53 pathway: positive and negative feedback loops. Oncogene, 24(17), 2899–2908.
35. Vogelstein, B., Lane, D., & Levine, A. J. (2000). Surfing the p53 network. Nature, 408(6810), 307–310
36. Jones, R. G., Plas, D. R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., Birnbaum, M. J., & Thompson, C. B. (2005). AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Molecular cell, 18(3), 283–293.
37. Sablina, A. A., Budanov, A. V., Ilyinskaya, G. V., Agapova, L. S., Kravchenko, J. E., & Chumakov, P. M. (2005). The antioxidant function of the p53 tumor suppressor. Nature medicine, 11(12), 1306–1313
38. Labuschagne, C. F., Zani, F., & Vousden, K. H. (2018). Control of metabolism by p53 - Cancer and beyond. Biochimica et biophysica acta. Reviews on cancer, 1870(1), 32–42.
39. Muller, P. A., & Vousden, K. H. (2013). p53 mutations in cancer. Nature cell biology, 15(1), 2–8.
40. Li, J., Yang, L., Gaur, S., Zhang, K., Wu, X., Yuan, Y. C., Li, H., Hu, S., Weng, Y., & Yen, Y. (2014). Mutants TP53 p.R273H and p.R273C but not p.R273G enhance cancer cell malignancy. Human mutation, 35(5), 575–584.
41. Sun, S., Chen, H., Sun, L., Wang, M., Wu, X., & Xiao, Z. J. (2020). Hotspot mutant p53-R273H inhibits KLF6 expression to promote cell migration and tumor metastasis. Cell death & disease, 11(7), 595.
42. Schulz-Heddergott, R., Stark, N., Edmunds, S. J., Li, J., Conradi, L. C., Bohnenberger, H., Ceteci, F., Greten, F. R., Dobbelstein, M., & Moll, U. M. (2018). Therapeutic Ablation of Gain-of-Function Mutant p53 in Colorectal Cancer Inhibits Stat3-Mediated Tumor Growth and Invasion. Cancer cell, 34(2), 298–314.
43. Klemke, L., Fehlau, C. F., Winkler, N., Toboll, F., Singh, S. K., Moll, U. M., & Schulz-Heddergott, R. (2021). The Gain-of-Function p53 R248W Mutant Promotes Migration by STAT3 Deregulation in Human Pancreatic Cancer Cells. Frontiers in oncology, 11, 642603.
44. Yu, X., Vazquez, A., Levine, A. J., & Carpizo, D. R. (2012). Allele-specific p53 mutant reactivation. Cancer cell, 21(5), 614–625.
45. Grugan, K. D., Vega, M. E., Wong, G. S., Diehl, J. A., Bass, A. J., Wong, K. K., Nakagawa, H., & Rustgi, A. K. (2013). A common p53 mutation (R175H) activates c-Met receptor tyrosine

kinase to enhance tumor cell invasion. Cancer biology & therapy, 14(9), 853–859.
46. Parry, D., & Peters, G. (1996). Temperature-sensitive mutants of p16CDKN2 associated with familial melanoma. Molecular and cellular biology, 16(7), 3844–3852.
47. Rutter, J. L., Goldstein, A. M., Dávila, M. R., Tucker, M. A., & Struewing, J. P. (2003). CDKN2A point mutations D153spl(c.457G>T) and IVS2+1G>T result in aberrant splice products affecting both p16INK4a and p14ARF. Oncogene, 22(28), 4444–4448.
48. Kannengiesser, C., Brookes, S., del Arroyo, A. G., Pham, D., Bombled, J., Barrois, M., Mauffret, O., Avril, M. F., Chompret, A., Lenoir, G. M., Sarasin, A., French Hereditary Melanoma Study Group, Peters, G., & Bressac-de Paillerets, B. (2009). Functional, structural, and genetic evaluation of 20 CDKN2A germ line mutations identified in melanoma-prone families or patients. Human mutation, 30(4), 564–574.
49. Easton, D. F., Pharoah, P. D., Antoniou, A. C., Tischkowitz, M., Tavtigian, S. V., Nathanson, K. L., Devilee, P., Meindl, A., Couch, F. J., Southey, M., Goldgar, D. E., Evans, D. G., Chenevix-Trench, G., Rahman, N., Robson, M., Domchek, S. M., & Foulkes, W. D. (2015). Gene-panel sequencing and the prediction of breast-cancer risk. The New England journal of medicine, 372(23), 2243–2257.
50. Griffith, O. L., Spies, N. C., Anurag, M., Griffith, M., Luo, J., Tu, D., Yeo, B., Kunisaki, J., Miller, C. A., Krysiak, K., Hundal, J., Ainscough, B. J., Skidmore, Z. L., Campbell, K., Kumar, R., Fronick, C., Cook, L., Snider, J. E., Davies, S., Kavuri, S. M., … Ellis, M. J. (2018). The prognostic effects of somatic mutations in ER-positive breast cancer. Nature communications, 9(1), 3476.
51. Nahar, R., Zhai, W., Zhang, T., Takano, A., Khng, A. J., Lee, Y. Y., Liu, X., Lim, C. H., Koh, T., Aung, Z. W., Lim, T., Veeravalli, L., Yuan, J., Teo, A., Chan, C. X., Poh, H. M., Chua, I., Liew, A. A., Lau, D., Kwang, X. L., … Tan, D. (2018). Elucidating the genomic architecture of Asian EGFR-mutant lung adenocarcinoma through multi-region exome sequencing. Nature communications, 9(1), 216.
52. Jette, N. R., Kumar, M., Radhamani, S., Arthur, G., Goutam, S., Yip, S., Kolinsky, M., Williams, G. J., Bose, P., & Lees-Miller, S. P. (2020). ATM-Deficient Cancers Provide New Opportunities for Precision Oncology. Cancers, 12(3), 687.
53. Duffy, M. J., Synnott, N. C., O'Grady, S., & Crown, J. (2020). Targeting p53 for the treatment of cancer. Seminars in cancer biology, S1044-579X(20)30160-7.
54. Zhu, G., Pan, C., Bei, J. X., Li, B., Liang, C., Xu, Y., & Fu, X. (2020). Mutant p53 in Cancer Progression and Targeted Therapies. Frontiers in oncology, 10, 595187.
55. Bykov, V., Eriksson, S. E., Bianchi, J., & Wiman, K. G. (2018). Targeting mutant p53 for efficient cancer therapy. Nature reviews. Cancer, 18(2), 89–102.
56. Kim E. S. (2017). Abemaciclib: First Global Approval. Drugs, 77(18), 2063–2070.
57. Dhillon S. (2015). Palbociclib: first global approval. Drugs, 75(5), 543–551.
58. Syed Y. Y. (2017). Ribociclib: First Global Approval. Drugs, 77(7), 799–807.
59. Dhillon S. (2021). Trilaciclib: First Approval. Drugs, 81(7), 867–874.
60. Gopalan, P., Pinder, M., Chiappori, A., Ivey, A.M., Villegas, A.G., & Kaye, F. (2014). A phase II clinical trial of the CDK 4/6 inhibitor palbociclib (PD 0332991) in previously treated, advanced non-small cell lung cancer (NSCLC) patients with inactivated CDKN2A. Journal of Clinical Oncology, 32, 8077-8077.
61. Young, R. J., Waldeck, K., Martin, C., Foo, J. H., Cameron, D. P., Kirby, L., Do, H., Mitchell, C., Cullinane, C., Liu, W., Fox, S. B., Dutton-Regester, K., Hayward, N. K., Jene, N., Dobrovic, A., Pearson, R. B., Christensen, J. G., Randolph, S., McArthur, G. A., & Sheppard, K. E. (2014). Loss of CDKN2A expression is a frequent event in primary invasive melanoma and correlates with sensitivity to the CDK4/6 inhibitor PD0332991 in melanoma cell lines. Pigment cell & melanoma research, 27(4), 590–600.
62. Su, D., Zhang, D., Jin, J., Ying, L., Han, M., Chen, K., Li, B., Wu, J., Xie, Z., Zhang, F., Lin, Y., Cheng, G., Li, J. Y., Huang, M., Wang, J., Wang, K., Zhang, J., Li, F., Xiong, L., Futreal, A., … Mao, W. (2019). Identification of predictors of drug sensitivity using patient-derived models of esophageal squamous cell carcinoma. Nature communications, 10(1), 5076.
63. Mateo, J., Carreira, S., Sandhu, S., Miranda, S., Mossop, H., Perez-Lopez, R., Nava Rodrigues, D., Robinson, D., Omlin, A., Tunariu, N., Boysen, G., Porta, N., Flohr, P., Gillman, A., Figueiredo, I., Paulding, C., Seed, G., Jain, S., Ralph, C., Protheroe, A., … de Bono, J. S.

(2015). DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. The New England journal of medicine, 373(18), 1697–1708.
64. Schmitt, A., Knittel, G., Welcker, D., Yang, T. P., George, J., Nowak, M., Leeser, U., Büttner, R., Perner, S., Peifer, M., & Reinhardt, H. C. (2017). ATM Deficiency Is Associated with Sensitivity to PARP1- and ATR Inhibitors in Lung Adenocarcinoma. Cancer research, 77(11), 3040–3056.
65. Lloyd, R. L., Wijnhoven, P., Ramos-Montoya, A., Wilson, Z., Illuzzi, G., Falenta, K., Jones, G. N., James, N., Chabbert, C. D., Stott, J., Dean, E., Lau, A., & Young, L. A. (2020). Combined PARP and ATR inhibition potentiates genome instability and cell death in ATM-deficient cancer cells. Oncogene, 39(25), 4869–4883.
66. Downward J. (2003). Targeting RAS signalling pathways in cancer therapy. Nature reviews. Cancer, 3(1), 11–22.
67. Kinbara, K., Goldfinger, L. E., Hansen, M., Chou, F. L., & Ginsberg, M. H. (2003). Ras GTPases: integrins' friends or foes?. Nature reviews. Molecular cell biology, 4(10), 767–776.
68. Molina, J. R., & Adjei, A. A. (2006). The Ras/Raf/MAPK pathway. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer, 1(1), 7–9.
69. Castellano, E., Molina-Arcas, M., Krygowska, A. A., East, P., Warne, P., Nicol, A., & Downward, J. (2016). RAS signalling through PI3-Kinase controls cell migration via modulation of Reelin expression. Nature communications, 7, 11245.
70. Imperial, R., Toor, O. M., Hussain, A., Subramanian, J., & Masood, A. (2019). Comprehensive pancancer genomic analysis reveals (RTK)-RAS-RAF-MEK as a key dysregulated pathway in cancer: Its clinical implications. Seminars in cancer biology, 54, 14–28.
71. Smith, M. J., Neel, B. G., & Ikura, M. (2013). NMR-based functional profiling of RASopathies and oncogenic RAS mutations. Proceedings of the National Academy of Sciences of the United States of America, 110(12), 4574–4579.
72. Cook, J. H., Melloni, G., Gulhan, D. C., Park, P. J., & Haigis, K. M. (2021). The origins and genetic interactions of KRAS mutations are allele- and tissue-specific. Nature communications, 12(1), 1808.
73. Feig, L. A., & Cooper, G. M. (1988). Relationship among guanine nucleotide exchange, GTP hydrolysis, and transforming potential of mutated ras proteins. Molecular and cellular biology, 8(6), 2472–2478.
74. Poulin, E. J., Bera, A. K., Lu, J., Lin, Y. J., Strasser, S. D., Paulo, J. A., Huang, T. Q., Morales, C., Yan, W., Cook, J., Nowak, J. A., Brubaker, D. K., Joughin, B. A., Johnson, C. W., DeStefanis, R. A., Ghazi, P. C., Gondi, S., Wales, T. E., Iacob, R. E., Bogdanova, L., … Haigis, K. M. (2019). Tissue-Specific Oncogenic Activity of KRASA146T. Cancer discovery, 9(6), 738–755.
75. Waters, A. M., & Der, C. J. (2018). KRAS: The Critical Driver and Therapeutic Target for Pancreatic Cancer. Cold Spring Harbor perspectives in medicine, 8(9), a031435.
76. Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M. J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., Menzies, A., … Futreal, P. A. (2002). Mutations of the BRAF gene in human cancer. Nature, 417(6892), 949–954.
77. Cantwell-Dorris, E. R., O'Leary, J. J., & Sheils, O. M. (2011). BRAFV600E: implications for carcinogenesis and molecular therapy. Molecular cancer therapeutics, 10(3), 385–394.
78. Falini, B., Martelli, M. P., & Tiacci, E. (2016). BRAF V600E mutation in hairy cell leukemia: from bench to bedside. Blood, 128(15), 1918–1927.
79. Ding, L., Getz, G., Wheeler, D. A., Mardis, E. R., McLellan, M. D., Cibulskis, K., Sougnez, C., Greulich, H., Muzny, D. M., Morgan, M. B., Fulton, L., Fulton, R. S., Zhang, Q., Wendl, M. C., Lawrence, M. S., Larson, D. E., Chen, K., Dooling, D. J., Sabo, A., Hawes, A. C., … Wilson, R. K. (2008). Somatic mutations affect key pathways in lung adenocarcinoma. Nature, 455(7216), 1069–1075.
80. Upadhyaya, M., Kluwe, L., Spurlock, G., Monem, B., Majounie, E., Mantripragada, K., Ruggieri, M., Chuzhanova, N., Evans, D. G., Ferner, R., Thomas, N., Guha, A., & Mautner, V. (2008). Germline and somatic NF1 gene mutation spectrum in NF1-associated malignant peripheral nerve sheath tumors (MPNSTs). Human mutation, 29(1), 74–82.
81. Brems, H., Beert, E., de Ravel, T., & Legius, E. (2009). Mechanisms in the pathogenesis of malignant tumours in neurofibromatosis type 1. The Lancet. Oncology, 10(5), 508–515.

82. Hodis, E., Watson, I. R., Kryukov, G. V., Arold, S. T., Imielinski, M., Theurillat, J. P., Nickerson, E., Auclair, D., Li, L., Place, C., Dicara, D., Ramos, A. H., Lawrence, M. S., Cibulskis, K., Sivachenko, A., Voet, D., Saksena, G., Stransky, N., Onofrio, R. C., Winckler, W., … Chin, L. (2012). A landscape of driver mutations in melanoma. Cell, 150(2), 251–263.
83. Dumbrava, E. I., & Meric-Bernstam, F. (2018). Personalized cancer therapy-leveraging a knowledge base for clinical decision-making. Cold Spring Harbor molecular case studies, 4(2), a001578.
84. Cox, A. D., Fesik, S. W., Kimmelman, A. C., Luo, J., & Der, C. J. (2014). Drugging the undruggable RAS: Mission possible?. Nature reviews. Drug discovery, 13(11), 828–851.
85. Keeton, A. B., Salter, E. A., & Piazza, G. A. (2017). The RAS-Effector Interaction as a Drug Target. Cancer research, 77(2), 221–226.
86. Liu, P., Wang, Y., & Li, X. (2019). Targeting the untargetable KRAS in cancer therapy. Acta pharmaceutica Sinica. B, 9(5), 871–879.
87. Sheridan C. (2020). Grail of RAS cancer drugs within reach. Nature biotechnology, 38(1), 6–8.
88. Blair H. A. (2021). Sotorasib: First Approval. Drugs, 81(13), 1573–1579.
89. G.J. Riely, S-H.I. Ou, I. Rybkin, A. Spira, K. Papadopoulos, J.K. Sabari, M. Johnson, R.S. Heist, L. Bazhenova, M. Barve, J.M. Pacheco, K. Velastegui, C. Cilliers, P. Olson, J.G. Christensen, T. Kheoh, R.C. Chao, P.A. Jänne. (2021). 99O_PR KRYSTAL-1: Activity and preliminary pharmacodynamic (PD) analysis of adagrasib (MRTX849) in patients (Pts) with advanced non–small cell lung cancer (NSCLC) harboring KRASG12C mutation. Journal of Thoracic Oncology, 16(4), 751-752.
90. Flaherty, K. T., Yasothan, U., & Kirkpatrick, P. (2011). Vemurafenib. Nature reviews. Drug discovery, 10(11), 811–812.
91. Ballantyne, A. D., & Garnock-Jones, K. P. (2013). Dabrafenib: first global approval. Drugs, 73(12), 1367–1376.
92. Shirley M. (2018). Encorafenib and Binimetinib: First Global Approvals. Drugs, 78(12), 1277–1284.
93. Garnock-Jones K. P. (2015). Cobimetinib: First Global Approval. Drugs, 75(15), 1823–1830.
94. Robert, C., Karaszewska, B., Schachter, J., Rutkowski, P., Mackiewicz, A., Stroiakovski, D., Lichinitser, M., Dummer, R., Grange, F., Mortier, L., Chiarion-Sileni, V., Drucis, K., Krajsova, I., Hauschild, A., Lorigan, P., Wolter, P., Long, G. V., Flaherty, K., Nathan, P., Ribas, A., … Schadendorf, D. (2015). Improved overall survival in melanoma with combined dabrafenib and trametinib. The New England journal of medicine, 372(1), 30–39.
95. Dummer, R., Ascierto, P. A., Gogas, H. J., Arance, A., Mandala, M., Liszkay, G., Garbe, C., Schadendorf, D., Krajsova, I., Gutzmer, R., Chiarion-Sileni, V., Dutriaux, C., de Groot, J., Yamazaki, N., Loquai, C., Moutouh-de Parseval, L. A., Pickard, M. D., Sandor, V., Robert, C., & Flaherty, K. T. (2018). Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial. The Lancet. Oncology, 19(5), 603–615.
96. Odogwu, L., Mathieu, L., Blumenthal, G., Larkins, E., Goldberg, K. B., Griffin, N., Bijwaard, K., Lee, E. Y., Philip, R., Jiang, X., Rodriguez, L., McKee, A. E., Keegan, P., & Pazdur, R. (2018). FDA Approval Summary: Dabrafenib and Trametinib for the Treatment of Metastatic Non-Small Cell Lung Cancers Harboring BRAF V600E Mutations. The oncologist, 23(6), 740–745.
97. Ascierto, P. A., Dummer, R., Gogas, H. J., Flaherty, K. T., Arance, A., Mandala, M., Liszkay, G., Garbe, C., Schadendorf, D., Krajsova, I., Gutzmer, R., de Groot, J., Loquai, C., Gollerkeri, A., Pickard, M. D., & Robert, C. (2020). Update on tolerability and overall survival in COLUMBUS: landmark analysis of a randomised phase 3 trial of encorafenib plus binimetinib vs vemurafenib or encorafenib in patients with BRAF V600-mutant melanoma. European journal of cancer (Oxford, England : 1990), 126, 33–44.
98. Salama, A., Li, S., Macrae, E. R., Park, J. I., Mitchell, E. P., Zwiebel, J. A., Chen, H. X., Gray, R. J., McShane, L. M., Rubinstein, L. V., Patton, D., Williams, P. M., Hamilton, S. R., Armstrong, D. K., Conley, B. A., Arteaga, C. L., Harris, L. N., O'Dwyer, P. J., Chen, A. P., & Flaherty, K. T. (2020). Dabrafenib and Trametinib in Patients With Tumors With BRAFV600E Mutations: Results of the NCI-MATCH Trial Subprotocol H. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 38(33), 3895–3904.
99. Zhou, D., Mattner, J., Cantu, C., 3rd, Schrantz, N., Yin, N., Gao, Y., Sagiv, Y., Hudspeth, K.,

Wu, Y. P., Yamashita, T., Teneberg, S., Wang, D., Proia, R. L., Levery, S. B., Savage, P. B., Teyton, L., & Bendelac, A. (2004). Lysosomal glycosphingolipid recognition by NKT cells. Science (New York, N.Y.), 306(5702), 1786–1789.
100. Groux-Degroote, S., van Dijk, S. M., Wolthoorn, J., Neumann, S., Theos, A. C., De Mazière, A. M., Klumperman, J., van Meer, G., & Sprong, H. (2008). Glycolipid-dependent sorting of melanosomal from lysosomal membrane proteins by lumenal determinants. Traffic (Copenhagen, Denmark), 9(6), 951–963.
101. Wang, D., & Dubois, R. N. (2010). Eicosanoids and cancer. Nature reviews. Cancer, 10(3), 181–193.
102. Hishikawa, D., Hashidate, T., Shimizu, T., & Shindou, H. (2014). Diversity and function of membrane glycerophospholipids generated by the remodeling pathway in mammalian cells. Journal of lipid research, 55(5), 799–807.
103. Gyamfi, D., E. Ofori Awuah, and S. Owusu (2019) in The Molecular Nutrition of Fats (Eds: V.B. Patel), Academic Press. p. 17-32.
104. DeBerardinis, R. J., Lum, J. J., Hatzivassiliou, G., & Thompson, C. B. (2008). The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell metabolism, 7(1), 11–20.
105. Vander Heiden, M. G., Cantley, L. C., & Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science (New York, N.Y.), 324(5930), 1029–1033.
106. Zhao, L., & Vogt, P. K. (2008). Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. Proceedings of the National Academy of Sciences of the United States of America, 105(7), 2652–2657.
107. Leontiadou, H., Galdadas, I., Athanasiou, C., & Cournia, Z. (2018). Insights into the mechanism of the PIK3CA E545K activating mutation using MD simulations. Scientific reports, 8(1), 15544.
108. Gkeka, P., Evangelidis, T., Pavlaki, M., Lazani, V., Christoforidis, S., Agianian, B., & Cournia, Z. (2014). Investigating the structure and dynamics of the PIK3CA wild-type and H1047R oncogenic mutant. PLoS computational biology, 10(10), e1003895.
109. Ikenoue, T., Kanai, F., Hikiba, Y., Obata, T., Tanaka, Y., Imamura, J., Ohta, M., Jazag, A., Guleng, B., Tateishi, K., Asaoka, Y., Matsumura, M., Kawabe, T., & Omata, M. (2005). Functional analysis of PIK3CA gene mutations in human colorectal cancer. Cancer research, 65(11), 4562–4567.
110. Kang, S., Bader, A. G., & Vogt, P. K. (2005). Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. Proceedings of the National Academy of Sciences of the United States of America, 102(3), 802–807.
111. Bader, A. G., Kang, S., & Vogt, P. K. (2006). Cancer-specific mutations in PIK3CA are oncogenic in vivo. Proceedings of the National Academy of Sciences of the United States of America, 103(5), 1475–1479.
112. Dogruluk, T., Tsang, Y. H., Espitia, M., Chen, F., Chen, T., Chong, Z., Appadurai, V., Dogruluk, A., Eterovic, A. K., Bonnen, P. E., Creighton, C. J., Chen, K., Mills, G. B., & Scott, K. L. (2015). Identification of Variant-Specific Functions of PIK3CA by Rapid Phenotyping of Rare Mutations. Cancer research, 75(24), 5341–5354.
113. Röhrig, F., & Schulze, A. (2016). The multifaceted roles of fatty acid synthesis in cancer. Nature reviews. Cancer, 16(11), 732–749.
114. Snaebjornsson, M. T., Janaki-Raman, S., & Schulze, A. (2020). Greasing the Wheels of the Cancer Machine: The Role of Lipid Metabolism in Cancer. Cell metabolism, 31(1), 62–76.
115. Georgescu, M. M., Kirsch, K. H., Akagi, T., Shishido, T., & Hanafusa, H. (1999). The tumor-suppressor activity of PTEN is regulated by its carboxyl-terminal region. Proceedings of the National Academy of Sciences of the United States of America, 96(18), 10182–10187.
116. Papa, A., Wan, L., Bonora, M., Salmena, L., Song, M. S., Hobbs, R. M., Lunardi, A., Webster, K., Ng, C., Newton, R. H., Knoblauch, N., Guarnerio, J., Ito, K., Turka, L. A., Beck, A. H., Pinton, P., Bronson, R. T., Wei, W., & Pandolfi, P. P. (2014). Cancer-associated PTEN mutants act in a dominant-negative manner to suppress PTEN protein function. Cell, 157(3), 595–610.
117. Rashmi, R., DeSelm, C., Helms, C., Bowcock, A., Rogers, B. E., Rader, J. L., Rader, J.,

Grigsby, P. W., & Schwarz, J. K. (2014). AKT inhibitors promote cell death in cervical cancer through disruption of mTOR signaling and glucose uptake. PloS one, 9(4), e92948.
118. Leslie, N. R., & den Hertog, J. (2014). Mutant PTEN in Cancer: Worse Than Nothing. Cell, 157(3), 527–529.
119. Salmena, L., Carracedo, A., & Pandolfi, P. P. (2008). Tenets of PTEN tumor suppression. Cell, 133(3), 403–414.
120. Choi, S. W., Lee, Y., Shin, K., Koo, H., Kim, D., Sa, J. K., Cho, H. J., Shin, H. M., Lee, S. J., Kim, H., Chung, S., Shin, J., Lee, C., & Nam, D. H. (2021). Mutation-specific non-canonical pathway of PTEN as a distinct therapeutic target for glioblastoma. Cell death & disease, 12(4), 374.
121. Kloosterhof, N. K., Bralten, L. B., Dubbink, H. J., French, P. J., & van den Bent, M. J. (2011). Isocitrate dehydrogenase-1 mutations: a fundamentally new understanding of diffuse glioma?. The Lancet. Oncology, 12(1), 83–91.
122. Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., Driggers, E. M., Fantin, V. R., Jang, H. G., Jin, S., Keenan, M. C., Marks, K. M., Prins, R. M., Ward, P. S., Yen, K. E., Liau, L. M., Rabinowitz, J. D., Cantley, L. C., Thompson, C. B., Vander Heiden, M. G., & Su, S. M. (2010). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature, 465(7300), 966.
123. Shim, E. H., Livi, C. B., Rakheja, D., Tan, J., Benson, D., Parekh, V., Kho, E. Y., Ghosh, A. P., Kirkman, R., Velu, S., Dutta, S., Chenna, B., Rea, S. L., Mishur, R. J., Li, Q., Johnson-Pais, T. L., Guo, L., Bae, S., Wei, S., Block, K., … Sudarshan, S. (2014). L-2-Hydroxyglutarate: an epigenetic modifier and putative oncometabolite in renal cancer. Cancer discovery, 4(11), 1290–1298.
124. Parker, S. J., & Metallo, C. M. (2015). Metabolic consequences of oncogenic IDH mutations. Pharmacology & therapeutics, 152, 54–62.
125. Clark, O., Yen, K., & Mellinghoff, I. K. (2016). Molecular Pathways: Isocitrate Dehydrogenase Mutations in Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research, 22(8), 1837–1842.
126. Yang, H., Ye, D., Guan, K. L., & Xiong, Y. (2012). IDH1 and IDH2 mutations in tumorigenesis: mechanistic insights and clinical perspectives. Clinical cancer research : an official journal of the American Association for Cancer Research, 18(20), 5562–5571.
127. Reitman, Z. J., Jin, G., Karoly, E. D., Spasojevic, I., Yang, J., Kinzler, K. W., He, Y., Bigner, D. D., Vogelstein, B., & Yan, H. (2011). Profiling the effects of isocitrate dehydrogenase 1 and 2 mutations on the cellular metabolome. Proceedings of the National Academy of Sciences of the United States of America, 108(8), 3270–3275.
128. Esmaeili, M., Hamans, B. C., Navis, A. C., van Horssen, R., Bathen, T. F., Gribbestad, I. S., Leenders, W. P., & Heerschap, A. (2014). IDH1 R132H mutation generates a distinct phospholipid metabolite profile in glioma. Cancer research, 74(17), 4898–4907.
129. Zhou, L., Wang, Z., Hu, C., Zhang, C., Kovatcheva-Datchary, P., Yu, D., Liu, S., Ren, F., Wang, X., Li, Y., Hou, X., Piao, H., Lu, X., Zhang, Y., & Xu, G. (2019). Integrated Metabolomics and Lipidomics Analyses Reveal Metabolic Reprogramming in Human Glioma with IDH1 Mutation. Journal of proteome research, 18(3), 960–969.
130. Hudes, G., Carducci, M., Tomczak, P., Dutcher, J., Figlin, R., Kapoor, A., Staroslawska, E., Sosman, J., McDermott, D., Bodrogi, I., Kovacevic, Z., Lesovoy, V., Schmidt-Wolf, I. G., Barbarash, O., Gokmen, E., O'Toole, T., Lustgarten, S., Moore, L., Motzer, R. J., & Global ARCC Trial (2007). Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. The New England journal of medicine, 356(22), 2271–2281.
131. Motzer, R. J., Escudier, B., Oudard, S., Hutson, T. E., Porta, C., Bracarda, S., Grünwald, V., Thompson, J. A., Figlin, R. A., Hollaender, N., Urbanowitz, G., Berg, W. J., Kay, A., Lebwohl, D., Ravaud, A., & RECORD-1 Study Group (2008). Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet (London, England), 372(9637), 449–456.
132. Benjamin, D., Colombi, M., Moroni, C., & Hall, M. N. (2011). Rapamycin passes the torch: a new generation of mTOR inhibitors. Nature reviews. Drug discovery, 10(11), 868–880.
133. Markham A. (2017). Copanlisib: First Global Approval. Drugs, 77(18), 2057–2062.
134. Hasskarl J. (2018). Everolimus. Recent results in cancer research. Fortschritte der

Krebsforschung. Progres dans les recherches sur le cancer, 211, 101–123.
135. Blair H. A. (2018). Duvelisib: First Global Approval. Drugs, 78(17), 1847–1853.
136. Markham A. (2019). Alpelisib: First Global Approval. Drugs, 79(11), 1249–1253.
137. Dhillon S. (2018). Ivosidenib: First Global Approval. Drugs, 78(14), 1509–1516.
138. Katso, R., Okkenhaug, K., Ahmadi, K., White, S., Timms, J., & Waterfield, M. D. (2001). Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. Annual review of cell and developmental biology, 17, 615–675.
139. Engelman, J. A., Luo, J., & Cantley, L. C. (2006). The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nature reviews. Genetics, 7(8), 606–619.
140. Courtney, K. D., Corcoran, R. B., & Engelman, J. A. (2010). The PI3K pathway as drug target in human cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 28(6), 1075–1083.
141. Fruman, D. A., Chiu, H., Hopkins, B. D., Bagrodia, S., Cantley, L. C., & Abraham, R. T. (2017). The PI3K Pathway in Human Disease. Cell, 170(4), 605–635.
142. Seton-Rogers S. (2014). Signalling: uncovering new functions of PI3K mutations. Nature reviews. Cancer, 14(12), 766–767.
143. Cheung, L. W., Yu, S., Zhang, D., Li, J., Ng, P. K., Panupinthu, N., Mitra, S., Ju, Z., Yu, Q., Liang, H., Hawke, D. H., Lu, Y., Broaddus, R. R., & Mills, G. B. (2014). Naturally occurring neomorphic PIK3R1 mutations activate the MAPK pathway, dictating therapeutic response to MAPK pathway inhibitors. Cancer cell, 26(4), 479–494.
144. Moukarzel, L. A., Da Cruz Paula, A., Ferrando, L., Hoang, T., Sebastiao, A., Pareja, F., Park, K. J., Jungbluth, A. A., Capella, G., Pineda, M., Levin, J. D., Abu-Rustum, N. R., Ellenson, L. H., Bel, A. V., Reis-Filho, J. S., Matias-Guiu, X., Cadoo, K., Stadler, Z. K., & Weigelt, B. (2021). Clonal relationship and directionality of progression of synchronous endometrial and ovarian carcinomas in patients with DNA mismatch repair-deficiency associated syndromes. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc, 34(5), 994–1007.
145. Sun, M., Hillmann, P., Hofmann, B. T., Hart, J. R., & Vogt, P. K. (2010). Cancer-derived mutations in the regulatory subunit p85alpha of phosphoinositide 3-kinase function through the catalytic subunit p110alpha. Proceedings of the National Academy of Sciences of the United States of America, 107(35), 15547–15552.
146. Li, X., Lau, A., Ng, A., Aldehaiman, A., Zhou, Y., Ng, P., Arold, S. T., & Cheung, L. (2021). Cancer-associated mutations in the p85α N-terminal SH2 domain activate a spectrum of receptor tyrosine kinases. Proceedings of the National Academy of Sciences of the United States of America, 118(37), e2101751118.
147. Song, M., Bode, A. M., Dong, Z., & Lee, M. H. (2019). AKT as a Therapeutic Target for Cancer. Cancer research, 79(6), 1019–1031.
148. Martorana, F., Motta, G., Pavone, G., Motta, L., Stella, S., Vitale, S. R., Manzella, L., & Vigneri, P. (2021). AKT Inhibitors: New Weapons in the Fight Against Breast Cancer?. Frontiers in pharmacology, 12, 662232.
149. Pickart C. M. (2000). Ubiquitin in chains. Trends in biochemical sciences, 25(11), 544–548.
150. Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J., Slaughter, C., Pickart, C., & Chen, Z. J. (2000). Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell, 103(2), 351–361.
151. Spence, J., Gali, R. R., Dittmar, G., Sherman, F., Karin, M., & Finley, D. (2000). Cell cycle-regulated modification of the ribosome by a variant multiubiquitin chain. Cell, 102(1), 67–76.
152. Hicke L. (2001). Protein regulation by monoubiquitin. Nature reviews. Molecular cell biology, 2(3), 195–201.
153. Katzmann, D. J., Odorizzi, G., & Emr, S. D. (2002). Receptor downregulation and multivesicular-body sorting. Nature reviews. Molecular cell biology, 3(12), 893–905.
154. Huen, M. S., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M. B., & Chen, J. (2007). RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell, 131(5), 901–914.
155. Mukhopadhyay, D., & Riezman, H. (2007). Proteasome-independent functions of ubiquitin in endocytosis and signaling. Science (New York, N.Y.), 315(5809), 201–205.
156. Yumimoto, K., Akiyoshi, S., Ueo, H., Sagara, Y., Onoyama, I., Ueo, H., Ohno, S., Mori, M.,

Mimori, K., & Nakayama, K. I. (2015). F-box protein FBXW7 inhibits cancer metastasis in a non-cell-autonomous manner. The Journal of clinical investigation, 125(2), 621–635.
157. Yeh, C. H., Bellon, M., & Nicot, C. (2018). FBXW7: a critical tumor suppressor of human cancers. Molecular cancer, 17(1), 115.
158. Close, V., Close, W., Kugler, S. J., Reichenzeller, M., Yosifov, D. Y., Bloehdorn, J., Pan, L., Tausch, E., Westhoff, M. A., Döhner, H., Stilgenbauer, S., Oswald, F., & Mertens, D. (2019). FBXW7 mutations reduce binding of NOTCH1, leading to cleaved NOTCH1 accumulation and target gene activation in CLL. Blood, 133(8), 830–839.
159. Oberg, C., Li, J., Pauley, A., Wolf, E., Gurney, M., & Lendahl, U. (2001). The Notch intracellular domain is ubiquitinated and negatively regulated by the mammalian Sel-10 homolog. The Journal of biological chemistry, 276(38), 35847–35853.
160. Koepp, D. M., Schaefer, L. K., Ye, X., Keyomarsi, K., Chu, C., Harper, J. W., & Elledge, S. J. (2001). Phosphorylation-dependent ubiquitination of cyclin E by the SCFFbw7 ubiquitin ligase. Science (New York, N.Y.), 294(5540), 173–177.
161. Yada, M., Hatakeyama, S., Kamura, T., Nishiyama, M., Tsunematsu, R., Imaki, H., Ishida, N., Okumura, F., Nakayama, K., & Nakayama, K. I. (2004). Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. The EMBO journal, 23(10), 2116–2125.
162. Wei, W., Jin, J., Schlisio, S., Harper, J. W., & Kaelin, W. G., Jr (2005). The v-Jun point mutation allows c-Jun to escape GSK3-dependent recognition and destruction by the Fbw7 ubiquitin ligase. Cancer cell, 8(1), 25–33.
163. Mao, J. H., Kim, I. J., Wu, D., Climent, J., Kang, H. C., DelRosario, R., & Balmain, A. (2008). FBXW7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. Science (New York, N.Y.), 321(5895), 1499–1502.
164. Inuzuka, H., Shaik, S., Onoyama, I., Gao, D., Tseng, A., Maser, R. S., Zhai, B., Wan, L., Gutierrez, A., Lau, A. W., Xiao, Y., Christie, A. L., Aster, J., Settleman, J., Gygi, S. P., Kung, A. L., Look, T., Nakayama, K. I., DePinho, R. A., & Wei, W. (2011). SCF(FBW7) regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. Nature, 471(7336), 104–109.
165. Liu, X., Wang, L., Zhao, K., Thompson, P. R., Hwang, Y., Marmorstein, R., & Cole, P. A. (2008). The structural basis of protein acetylation by the p300/CBP transcriptional coactivator. Nature, 451(7180), 846–850.
166. Delvecchio, M., Gaucher, J., Aguilar-Gurrieri, C., Ortega, E., & Panne, D. (2013). Structure of the p300 catalytic core and implications for chromatin targeting and HAT regulation. Nature structural & molecular biology, 20(9), 1040–1046.
167. Pao, G. M., Janknecht, R., Ruffner, H., Hunter, T., & Verma, I. M. (2000). CBP/p300 interact with and function as transcriptional coactivators of BRCA1. Proceedings of the National Academy of Sciences of the United States of America, 97(3), 1020–1025.
168. Chan, H. M., Krstic-Demonacos, M., Smith, L., Demonacos, C., & La Thangue, N. B. (2001). Acetylation control of the retinoblastoma tumour-suppressor protein. Nature cell biology, 3(7), 667–674.
169. Grossman S. R. (2001). p300/CBP/p53 interaction and regulation of the p53 response. European journal of biochemistry, 268(10), 2773–2778.
170. Friedrichs, N., Jäger, R., Paggen, E., Rudlowski, C., Merkelbach-Bruse, S., Schorle, H., & Buettner, R. (2005). Distinct spatial expression patterns of AP-2alpha and AP-2gamma in non-neoplastic human breast and breast cancer. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc, 18(3), 431–438.
171. Salloum, R., McConechy, M. K., Mikael, L. G., Fuller, C., Drissi, R., DeWire, M., Nikbakht, H., De Jay, N., Yang, X., Boue, D., Chow, L., Finlay, J. L., Gayden, T., Karamchandani, J., Hummel, T. R., Olshefski, R., Osorio, D. S., Stevenson, C., Kleinman, C. L., Majewski, J., … Jabado, N. (2017). Characterizing temporal genomic heterogeneity in pediatric high-grade gliomas. Acta neuropathologica communications, 5(1), 78.
172. Shi, D., Pop, M. S., Kulikov, R., Love, I. M., Kung, A. L., & Grossman, S. R. (2009). CBP and p300 are cytoplasmic E4 polyubiquitin ligases for p53. Proceedings of the National Academy of Sciences of the United States of America, 106(38), 16275–16280.
173. Attar, N., & Kurdistani, S. K. (2017). Exploitation of EP300 and CREBBP Lysine Acetyltransferases by Cancer. Cold Spring Harbor perspectives in medicine, 7(3), a026534.

174. Pasqualucci, L., Dominguez-Sola, D., Chiarenza, A., Fabbri, G., Grunn, A., Trifonov, V., Kasper, L. H., Lerach, S., Tang, H., Ma, J., Rossi, D., Chadburn, A., Murty, V. V., Mullighan, C. G., Gaidano, G., Rabadan, R., Brindle, P. K., & Dalla-Favera, R. (2011). Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature, 471(7337), 189–195.
175. Peifer, M., Fernández-Cuesta, L., Sos, M. L., George, J., Seidel, D., Kasper, L. H., Plenker, D., Leenders, F., Sun, R., Zander, T., Menon, R., Koker, M., Dahmen, I., Müller, C., Di Cerbo, V., Schildhaus, H. U., Altmüller, J., Baessmann, I., Becker, C., de Wilde, B., … Thomas, R. K. (2012). Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nature genetics, 44(10), 1104–1110.
176. Merk, D. J., Ohli, J., Merk, N. D., Thatikonda, V., Morrissy, S., Schoof, M., Schmid, S. N., Harrison, L., Filser, S., Ahlfeld, J., Erkek, S., Raithatha, K., Andreska, T., Weißhaar, M., Launspach, M., Neumann, J. E., Shakarami, M., Plenker, D., Marra, M. A., Li, Y., … Schüller, U. (2018). Opposing Effects of CREBBP Mutations Govern the Phenotype of Rubinstein-Taybi Syndrome and Adult SHH Medulloblastoma. Developmental cell, 44(6), 709–724.e6.
177. Mondello, P., Tadros, S., Teater, M., Fontan, L., Chang, A. Y., Jain, N., Yang, H., Singh, S., Ying, H. Y., Chu, C. S., Ma, M., Toska, E., Alig, S., Durant, M., de Stanchina, E., Ghosh, S., Mottok, A., Nastoupil, L., Neelapu, S. S., Weigert, O., … Green, M. R. (2020). Selective Inhibition of HDAC3 Targets Synthetic Vulnerabilities and Activates Immune Surveillance in Lymphoma. Cancer discovery, 10(3), 440–459.
178. Deng, L., Meng, T., Chen, L., Wei, W., & Wang, P. (2020). The role of ubiquitination in tumorigenesis and targeted drug discovery. Signal transduction and targeted therapy, 5(1), 11.
179. Lu, G., Middleton, R. E., Sun, H., Naniong, M., Ott, C. J., Mitsiades, C. S., Wong, K. K., Bradner, J. E., & Kaelin, W. G., Jr (2014). The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. Science (New York, N.Y.), 343(6168), 305–309.
180. Stewart A. K. (2014). Medicine. How thalidomide works against cancer. Science (New York, N.Y.), 343(6168), 256–257.
181. Fricker L. D. (2020). Proteasome Inhibitor Drugs. Annual review of pharmacology and toxicology, 60, 457–476.
182. Dick, L. R., & Fleming, P. E. (2010). Building on bortezomib: second-generation proteasome inhibitors as anti-cancer therapy. Drug discovery today, 15(5-6), 243–249.
183. Nelson, W. J., & Nusse, R. (2004). Convergence of Wnt, beta-catenin, and cadherin pathways. Science (New York, N.Y.), 303(5663), 1483–1487.
184. Gattinoni, L., Zhong, X. S., Palmer, D. C., Ji, Y., Hinrichs, C. S., Yu, Z., Wrzesinski, C., Boni, A., Cassard, L., Garvin, L. M., Paulos, C. M., Muranski, P., & Restifo, N. P. (2009). Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. Nature medicine, 15(7), 808–813.
185. Petersen, C. P., & Reddien, P. W. (2009). Wnt signaling and the polarity of the primary body axis. Cell, 139(6), 1056–1068.
186. Spranger, S., & Gajewski, T. F. (2015). A new paradigm for tumor immune escape: β-catenin-driven immune exclusion. Journal for immunotherapy of cancer, 3, 43.
187. Junge H. J. (2017). Ligand-Selective Wnt Receptor Complexes in CNS Blood Vessels: RECK and GPR124 Plugged In. Neuron, 95(5), 983–985.
188. Miyoshi, Y., Nagase, H., Ando, H., Horii, A., Ichii, S., Nakatsuru, S., Aoki, T., Miki, Y., Mori, T., & Nakamura, Y. (1992). Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. Human molecular genetics, 1(4), 229–233.
189. Fearnhead, N. S., Britton, M. P., & Bodmer, W. F. (2001). The ABC of APC. Human molecular genetics, 10(7), 721–733.
190. Azzopardi, D., Dallosso, A. R., Eliason, K., Hendrickson, B. C., Jones, N., Rawstorne, E., Colley, J., Moskvina, V., Frye, C., Sampson, J. R., Wenstrup, R., Scholl, T., & Cheadle, J. P. (2008). Multiple rare nonsynonymous variants in the adenomatous polyposis coli gene predispose to colorectal adenomas. Cancer research, 68(2), 358–363.
191. Pai, S. G., Carneiro, B. A., Mota, J. M., Costa, R., Leite, C. A., Barroso-Sousa, R., Kaplan, J. B., Chae, Y. K., & Giles, F. J. (2017). Wnt/beta-catenin pathway: modulating anticancer immune response. Journal of hematology & oncology, 10(1), 101.
192. Imperial, R., Ahmed, Z., Toor, O. M., Erdoğan, C., Khaliq, A., Case, P., Case, J., Kennedy,

K., Cummings, L. S., Melton, N., Raza, S., Diri, B., Mohammad, R., El-Rayes, B., Pluard, T., Hussain, A., Subramanian, J., & Masood, A. (2018). Comparative proteogenomic analysis of right-sided colon cancer, left-sided colon cancer and rectal cancer reveals distinct mutational profiles. Molecular cancer, 17(1), 177.
193. Ficari, F., Cama, A., Valanzano, R., Curia, M. C., Palmirotta, R., Aceto, G., Esposito, D. L., Crognale, S., Lombardi, A., Messerini, L., Mariani-Costantini, R., Tonelli, F., & Battista, P. (2000). APC gene mutations and colorectal adenomatosis in familial adenomatous polyposis. British journal of cancer, 82(2), 348–353.
194. Mihalatos, M., Danielides, I., Belogianni, J., Harokopos, E., Papadopoulou, E., Kalimanis, G., Tsiava, M., Triantafillidis, J. K., Kosmidis, P. A., Fountzilas, G., Basdanis, G., Agnantis, N. J., Yannoukakos, D., & Nasioulas, G. (2003). Novel mutations of the APC gene in familial adenomatous polyposis in Greek patients. Cancer genetics and cytogenetics, 141(1), 65–70.
195. Kitagawa, M., Hatakeyama, S., Shirane, M., Matsumoto, M., Ishida, N., Hattori, K., Nakamichi, I., Kikuchi, A., Nakayama, K., & Nakayama, K. (1999). An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of beta-catenin. The EMBO journal, 18(9), 2401–2410.
196. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., & Kinzler, K. W. (1997). Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science (New York, N.Y.), 275(5307), 1787–1790.
197. Rubinfeld, B., Robbins, P., El-Gamil, M., Albert, I., Porfiri, E., & Polakis, P. (1997). Stabilization of beta-catenin by genetic defects in melanoma cell lines. Science (New York, N.Y.), 275(5307), 1790–1792.
198. Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G. H., Tan, Y., Zhang, Z., Lin, X., & He, X. (2002). Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell, 108(6), 837–847.
199. Kikuchi A. (2003). Tumor formation by genetic mutations in the components of the Wnt signaling pathway. Cancer science, 94(3), 225–229.
200. Rebouissou, S., Franconi, A., Calderaro, J., Letouzé, E., Imbeaud, S., Pilati, C., Nault, J. C., Couchy, G., Laurent, A., Balabaud, C., Bioulac-Sage, P., & Zucman-Rossi, J. (2016). Genotype-phenotype correlation of CTNNB1 mutations reveals different ß-catenin activity associated with liver tumor progression. Hepatology (Baltimore, Md.), 64(6), 2047–2061.
201. Tu, J., Park, S., Yu, W., Zhang, S., Wu, L., Carmon, K., & Liu, Q. J. (2019). The most common RNF43 mutant G659Vfs*41 is fully functional in inhibiting Wnt signaling and unlikely to play a role in tumorigenesis. Scientific reports, 9(1), 18557.
202. Koo, B. K., Spit, M., Jordens, I., Low, T. Y., Stange, D. E., van de Wetering, M., van Es, J. H., Mohammed, S., Heck, A. J., Maurice, M. M., & Clevers, H. (2012). Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. Nature, 488(7413), 665–669.
203. Hao, H. X., Xie, Y., Zhang, Y., Charlat, O., Oster, E., Avello, M., Lei, H., Mickanin, C., Liu, D., Ruffner, H., Mao, X., Ma, Q., Zamponi, R., Bouwmeester, T., Finan, P. M., Kirschner, M. W., Porter, J. A., Serluca, F. C., & Cong, F. (2012). ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. Nature, 485(7397), 195–200.
204. UniProt Consortium (2021). UniProt: the universal protein knowledgebase in 2021. Nucleic acids research, 49(D1), D480–D489.
205. Spit, M., Fenderico, N., Jordens, I., Radaszkiewicz, T., Lindeboom, R. G., Bugter, J. M., Cristobal, A., Ootes, L., van Osch, M., Janssen, E., Boonekamp, K. E., Hanakova, K., Potesil, D., Zdrahal, Z., Boj, S. F., Medema, J. P., Bryja, V., Koo, B. K., Vermeulen, M., & Maurice, M. M. (2020). RNF43 truncations trap CK1 to drive niche-independent self-renewal in cancer. The EMBO journal, 39(18), e103932.
206. Anastas, J. N., & Moon, R. T. (2013). WNT signalling pathways as therapeutic targets in cancer. Nature reviews. Cancer, 13(1), 11–26.
207. Jung, Y. S., & Park, J. I. (2020). Wnt signaling in cancer: therapeutic targeting of Wnt signaling beyond β-catenin and the destruction complex. Experimental & molecular medicine, 52(2), 183–191.
208. Hori, K., Sen, A., & Artavanis-Tsakonas, S. (2013). Notch signaling at a glance. Journal of cell science, 126(Pt 10), 2135–2140.

209. Bray S. J. (2016). Notch signalling in context. Nature reviews. Molecular cell biology, 17(11), 722–735.
210. Siebel, C., & Lendahl, U. (2017). Notch Signaling in Development, Tissue Homeostasis, and Disease. Physiological reviews, 97(4), 1235–1294.
211. Yeh, C. H., Bellon, M., Pancewicz-Wojtkiewicz, J., & Nicot, C. (2016). Oncogenic mutations in the FBXW7 gene of adult T-cell leukemia patients. Proceedings of the National Academy of Sciences of the United States of America, 113(24), 6731–6736.
212. Pancewicz, J., Taylor, J. M., Datta, A., Baydoun, H. H., Waldmann, T. A., Hermine, O., & Nicot, C. (2010). Notch signaling contributes to proliferation and tumor formation of human T-cell leukemia virus type 1-associated adult T-cell leukemia. Proceedings of the National Academy of Sciences of the United States of America, 107(38), 16619–16624.
213. Oswald, F., Täuber, B., Dobner, T., Bourteele, S., Kostezka, U., Adler, G., Liptay, S., & Schmid, R. M. (2001). p300 acts as a transcriptional coactivator for mammalian Notch-1. Molecular and cellular biology, 21(22), 7761–7774.
214. Wallberg, A. E., Pedersen, K., Lendahl, U., & Roeder, R. G. (2002). p300 and PCAF act cooperatively to mediate transcriptional activation from chromatin templates by notch intracellular domains in vitro. Molecular and cellular biology, 22(22), 7812–7819.
215. Huang, Y. H., Cai, K., Xu, P. P., Wang, L., Huang, C. X., Fang, Y., Cheng, S., Sun, X. J., Liu, F., Huang, J. Y., Ji, M. M., & Zhao, W. L. (2021). CREBBP/EP300 mutations promoted tumor progression in diffuse large B-cell lymphoma through altering tumor-associated macrophage polarization via FBXW7-NOTCH-CCL2/CSF1 axis. Signal transduction and targeted therapy, 6(1), 10.
216. Majumder, S., Crabtree, J. S., Golde, T. E., Minter, L. M., Osborne, B. A., & Miele, L. (2021). Targeting Notch in oncology: the path forward. Nature reviews. Drug discovery, 20(2), 125–144.
217. Pannuti, A., Foreman, K., Rizzo, P., Osipo, C., Golde, T., Osborne, B., & Miele, L. (2010). Targeting Notch to target cancer stem cells. Clinical cancer research : an official journal of the American Association for Cancer Research, 16(12), 3141–3152.
218. Takebe, N., Nguyen, D., & Yang, S. X. (2014). Targeting notch signaling pathway in cancer: clinical development advances and challenges. Pharmacology & therapeutics, 141(2), 140–149.
219. Malumbres, M., & Barbacid, M. (2009). Cell cycle, CDKs and cancer: a changing paradigm. Nature reviews. Cancer, 9(3), 153–166.
220. Matthews, H. K., Bertoli, C., & de Bruin, R. (2021). Cell cycle control in cancer. Nature reviews. Molecular cell biology, 10.1038/s41580-021-00404-3.
221. Cowell, J. K., Smith, T., & Bia, B. (1994). Frequent constitutional C to T mutations in CGA-arginine codons in the RB1 gene produce premature stop codons in patients with bilateral (hereditary) retinoblastoma. European journal of human genetics : EJHG, 2(4), 281–290.
222. Richter, S., Vandezande, K., Chen, N., Zhang, K., Sutherland, J., Anderson, J., Han, L., Panton, R., Branco, P., & Gallie, B. (2003). Sensitive and efficient detection of RB1 gene mutations enhances care for families with retinoblastoma. American journal of human genetics, 72(2), 253–269.
223. Ayari Jeridi, H., Bouguila, H., Ansperger-Rescher, B., Baroudi, O., Mdimegh, I., Omran, I., Charradi, K., Bouzayene, H., Benammar-Elgaaïed, A., & Lohmann, D. R. (2014). Genetic testing in Tunisian families with heritable retinoblastoma using a low cost approach permits accurate risk prediction in relatives and reveals incomplete penetrance in adults. Experimental eye research, 124, 48–55.
224. Yu, H. A., Suzawa, K., Jordan, E., Zehir, A., Ni, A., Kim, R., Kris, M. G., Hellmann, M. D., Li, B. T., Somwar, R., Solit, D. B., Berger, M. F., Arcila, M., Riely, G. J., & Ladanyi, M. (2018). Concurrent Alterations in EGFR-Mutant Lung Cancers Associated with Resistance to EGFR Kinase Inhibitors and Characterization of MTOR as a Mediator of Resistance. Clinical cancer research : an official journal of the American Association for Cancer Research, 24(13), 3108–3118.
225. Trimarchi, J. M., & Lees, J. A. (2002). Sibling rivalry in the E2F family. Nature reviews. Molecular cell biology, 3(1), 11–20.
226. Burkhart, D. L., & Sage, J. (2008). Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nature reviews. Cancer, 8(9), 671–682.

227. Xiong, Y., Hannon, G. J., Zhang, H., Casso, D., Kobayashi, R., & Beach, D. (1993). p21 is a universal inhibitor of cyclin kinases. Nature, 366(6456), 701–704.
228. Waga, S., Hannon, G. J., Beach, D., & Stillman, B. (1994). The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. Nature, 369(6481), 574–578.
229. Jackson, R. J., Adnane, J., Coppola, D., Cantor, A., Sebti, S. M., & Pledger, W. J. (2002). Loss of the cell cycle inhibitors p21(Cip1) and p27(Kip1) enhances tumorigenesis in knockout mouse models. Oncogene, 21(55), 8486–8497.
230. Poole, A. J., Heap, D., Carroll, R. E., & Tyner, A. L. (2004). Tumor suppressor functions for the Cdk inhibitor p21 in the mouse colon. Oncogene, 23(49), 8128–8134.
231. Forster, K., Obermeier, A., Mitina, O., Simon, N., Warmuth, M., Krause, G., & Hallek, M. (2008). Role of p21(WAF1/CIP1) as an attenuator of both proliferative and drug-induced apoptotic signals in BCR-ABL-transformed hematopoietic cells. Annals of hematology, 87(3), 183–193.
232. Suzuki, A., Tsutomi, Y., Miura, M., & Akahane, K. (1999). Caspase 3 inactivation to suppress Fas-mediated apoptosis: identification of binding domain with p21 and ILP and inactivation machinery by p21. Oncogene, 18(5), 1239–1244.
233. Yu, F., Megyesi, J., Safirstein, R. L., & Price, P. M. (2005). Identification of the functional domain of p21(WAF1/CIP1) that protects cells from cisplatin cytotoxicity. American journal of physiology. Renal physiology, 289(3), F514–F520.
234. Suski, J. M., Braun, M., Strmiska, V., & Sicinski, P. (2021). Targeting cell-cycle machinery in cancer. Cancer cell, 39(6), 759–778.
235. Otto, T., & Sicinski, P. (2017). Cell cycle proteins as promising targets in cancer therapy. Nature reviews. Cancer, 17(2), 93–115.
236. Karran P. (2000). DNA double strand break repair in mammalian cells. Current opinion in genetics & development, 10(2), 144–150.
237. Chen, C. C., Feng, W., Lim, P. X., Kass, E. M., & Jasin, M. (2018). Homology-Directed Repair and the Role of BRCA1, BRCA2, and Related Proteins in Genome Integrity and Cancer. Annual review of cancer biology, 2, 313–336.
238. van Gent, D. C., Hoeijmakers, J. H., & Kanaar, R. (2001). Chromosomal stability and the DNA double-stranded break connection. Nature reviews. Genetics, 2(3), 196–206.
239. Khanna, K. K., & Jackson, S. P. (2001). DNA double-strand breaks: signaling, repair and the cancer connection. Nature genetics, 27(3), 247–254.
240. Caburet, S., Heddar, A., Dardillac, E., Creux, H., Lambert, M., Messiaen, S., Tourpin, S., Livera, G., Lopez, B. S., & Misrahi, M. (2020). Homozygous hypomorphic BRCA2 variant in primary ovarian insufficiency without cancer or Fanconi anaemia trait. Journal of medical genetics, jmedgenet-2019-106672.
241. Sun, P., Li, Y., Chao, X., Li, J., Luo, R., Li, M., & He, J. (2020). Clinical characteristics and prognostic implications of BRCA-associated tumors in males: a pan-tumor survey. BMC cancer, 20(1), 994.
242. Lee H. (2014). Cycling with BRCA2 from DNA repair to mitosis. Experimental cell research, 329(1), 78–84.
243. Moynahan, M. E., Pierce, A. J., & Jasin, M. (2001). BRCA2 is required for homology-directed repair of chromosomal breaks. Molecular cell, 7(2), 263–272.
244. Liu, J., Doty, T., Gibson, B., & Heyer, W. D. (2010). Human BRCA2 protein promotes RAD51 filament formation on RPA-covered single-stranded DNA. Nature structural & molecular biology, 17(10), 1260–1262.
245. Tutt, A., Bertwistle, D., Valentine, J., Gabriel, A., Swift, S., Ross, G., Griffin, C., Thacker, J., & Ashworth, A. (2001). Mutation in Brca2 stimulates error-prone homology-directed repair of DNA double-strand breaks occurring between repeated sequences. The EMBO journal, 20(17), 4704–4716.
246. Rebbeck, T. R., Friebel, T. M., Friedman, E., Hamann, U., Huo, D., Kwong, A., Olah, E., Olopade, O. I., Solano, A. R., Teo, S. H., Thomassen, M., Weitzel, J. N., Chan, T. L., Couch, F. J., Goldgar, D. E., Kruse, T. A., Palmero, E. I., Park, S. K., Torres, D., van Rensburg, E. J., … Nathanson, K. L. (2018). Mutational spectrum in a worldwide study of 29,700 families with BRCA1 or BRCA2 mutations. Human mutation, 39(5), 593–620.

247. Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., & Livingston, D. M. (1997). Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell, 88(2), 265–275.
248. Zhong, Q., Chen, C. F., Li, S., Chen, Y., Wang, C. C., Xiao, J., Chen, P. L., Sharp, Z. D., & Lee, W. H. (1999). Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. Science (New York, N.Y.), 285(5428), 747–750.
249. Clark, S. L., Rodriguez, A. M., Snyder, R. R., Hankins, G. D., & Boehning, D. (2012). Structure-Function Of The Tumor Suppressor BRCA1. Computational and structural biotechnology journal, 1(1), e201204005.
250. Christou, C. M., & Kyriacou, K. (2013). BRCA1 and Its Network of Interacting Partners. Biology, 2(1), 40–63.
251. Bunting, S. F., Callén, E., Wong, N., Chen, H. T., Polato, F., Gunn, A., Bothmer, A., Feldhahn, N., Fernandez-Capetillo, O., Cao, L., Xu, X., Deng, C. X., Finkel, T., Nussenzweig, M., Stark, J. M., & Nussenzweig, A. (2010). 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell, 141(2), 243–254.
252. Zhang, J., Yan, Z., Wang, Y., Wang, Y., Guo, X., Jing, J., Dong, X., Dong, S., Liu, X., Yu, X., & Wu, C. (2021). Cancer-associated 53BP1 mutations induce DNA damage repair defects. Cancer letters, 501, 43–54.
253. Bunting, S. F., Callén, E., Wong, N., Chen, H. T., Polato, F., Gunn, A., Bothmer, A., Feldhahn, N., Fernandez-Capetillo, O., Cao, L., Xu, X., Deng, C. X., Finkel, T., Nussenzweig, M., Stark, J. M., & Nussenzweig, A. (2010). 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell, 141(2), 243–254.
254. Panier, S., & Boulton, S. J. (2014). Double-strand break repair: 53BP1 comes into focus. Nature reviews. Molecular cell biology, 15(1), 7–18.
255. Mao, Z., Bozzella, M., Seluanov, A., & Gorbunova, V. (2008). DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. Cell cycle (Georgetown, Tex.), 7(18), 2902–2906.
256. Yap, T. A., Sandhu, S. K., Carden, C. P., & de Bono, J. S. (2011). Poly(ADP-ribose) polymerase (PARP) inhibitors: Exploiting a synthetic lethal strategy in the clinic. CA: a cancer journal for clinicians, 61(1), 31–49.
257. Furgason, J. M., & Bahassi, e. (2013). Targeting DNA repair mechanisms in cancer. Pharmacology & therapeutics, 137(3), 298–308.
258. Brown, J. S., O'Carrigan, B., Jackson, S. P., & Yap, T. A. (2017). Targeting DNA Repair in Cancer: Beyond PARP Inhibitors. Cancer discovery, 7(1), 20–37.
259. Helleday, T., Petermann, E., Lundin, C., Hodgson, B., & Sharma, R. A. (2008). DNA repair pathways as targets for cancer therapy. Nature reviews. Cancer, 8(3), 193–204.
260. Deeks E. D. (2015). Olaparib: first global approval. Drugs, 75(2), 231–240.
261. Syed Y. Y. (2017). Rucaparib: First Global Approval. Drugs, 77(5), 585–592.
262. Scott L. J. (2017). Niraparib: First Global Approval. Drugs, 77(9), 1029–1034.
263. Hoy S. M. (2018). Talazoparib: First Global Approval. Drugs, 78(18), 1939–1946.
264. Arora, S., Balasubramaniam, S., Zhang, H., Berman, T., Narayan, P., Suzman, D., Bloomquist, E., Tang, S., Gong, Y., Sridhara, R., Turcu, F. R., Chatterjee, D., Saritas-Yildirim, B., Ghosh, S., Philip, R., Pathak, A., Gao, J. J., Amiri-Kordestani, L., Pazdur, R., & Beaver, J. A. (2021). FDA Approval Summary: Olaparib Monotherapy or in Combination with Bevacizumab for the Maintenance Treatment of Patients with Advanced Ovarian Cancer. The oncologist, 26(1), e164–e172.
265. Kwok, M., Davies, N., Agathanggelou, A., Smith, E., Oldreive, C., Petermann, E., Stewart, G., Brown, J., Lau, A., Pratt, G., Parry, H., Taylor, M., Moss, P., Hillmen, P., & Stankovic, T. (2016). ATR inhibition induces synthetic lethality and overcomes chemoresistance in TP53- or ATM-defective chronic lymphocytic leukemia cells. Blood, 127(5), 582–595.
266. Menolfi, D., & Zha, S. (2020). ATM, ATR and DNA-PKcs kinases-the lessons from the mouse models: inhibition ≠ deletion. Cell & bioscience, 10, 8.
267. Topatana, W., Juengpanich, S., Li, S., Cao, J., Hu, J., Lee, J., Suliyanto, K., Ma, D., Zhang, B., Chen, M., & Cai, X. (2020). Advances in synthetic lethality for cancer therapy: cellular mechanism and clinical translation. Journal of hematology & oncology, 13(1), 118.
268. David, C. J., & Manley, J. L. (2010). Alternative pre-mRNA splicing regulation in cancer:

pathways and programs unhinged. Genes & development, 24(21), 2343–2364.
269. Dvinge, H., Kim, E., Abdel-Wahab, O., & Bradley, R. K. (2016). RNA splicing factors as oncoproteins and tumour suppressors. Nature reviews. Cancer, 16(7), 413–430.
270. Climente-González, H., Porta-Pardo, E., Godzik, A., & Eyras, E. (2017). The Functional Impact of Alternative Splicing in Cancer. Cell reports, 20(9), 2215–2226.
271. Rahman, M. A., Krainer, A. R., & Abdel-Wahab, O. (2020). SnapShot: Splicing Alterations in Cancer. Cell, 180(1), 208–208.
272. Légaré, S., Cavallone, L., Mamo, A., Chabot, C., Sirois, I., Magliocco, A., Klimowicz, A., Tonin, P. N., Buchanan, M., Keilty, D., Hassan, S., Laperrière, D., Mader, S., Aleynikova, O., & Basik, M. (2015). The Estrogen Receptor Cofactor SPEN Functions as a Tumor Suppressor and Candidate Biomarker of Drug Responsiveness in Hormone-Dependent Breast Cancers. Cancer research, 75(20), 4351–4363.
273. Ariyoshi, M., & Schwabe, J. W. (2003). A conserved structural motif reveals the essential transcriptional repression function of Spen proteins and their role in developmental signaling. Genes & development, 17(15), 1909–1920.
274. Hiriart, E., Gruffat, H., Buisson, M., Mikaelian, I., Keppler, S., Meresse, P., Mercher, T., Bernard, O. A., Sergeant, A., & Manet, E. (2005). Interaction of the Epstein-Barr virus mRNA export factor EB2 with human Spen proteins SHARP, OTT1, and a novel member of the family, OTT3, links Spen proteins with splicing regulation and mRNA export. The Journal of biological chemistry, 280(44), 36935–36945.
275. Bartkowiak, B., & Greenleaf, A. L. (2015). Expression, purification, and identification of associated proteins of the full-length hCDK12/CyclinK complex. The Journal of biological chemistry, 290(3), 1786–1795.
276. Ekumi, K. M., Paculova, H., Lenasi, T., Pospichalova, V., Bösken, C. A., Rybarikova, J., Bryja, V., Geyer, M., Blazek, D., & Barboric, M. (2015). Ovarian carcinoma CDK12 mutations misregulate expression of DNA repair genes via deficient formation and function of the Cdk12/CycK complex. Nucleic acids research, 43(5), 2575–2589.
277. Blazek, D., Kohoutek, J., Bartholomeeusen, K., Johansen, E., Hulinkova, P., Luo, Z., Cimermancic, P., Ule, J., & Peterlin, B. M. (2011). The Cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes. Genes & development, 25(20), 2158–2172.
278. Krajewska, M., Dries, R., Grassetti, A. V., Dust, S., Gao, Y., Huang, H., Sharma, B., Day, D. S., Kwiatkowski, N., Pomaville, M., Dodd, O., Chipumuro, E., Zhang, T., Greenleaf, A. L., Yuan, G. C., Gray, N. S., Young, R. A., Geyer, M., Gerber, S. A., & George, R. E. (2019). CDK12 loss in cancer cells affects DNA damage response genes through premature cleavage and polyadenylation. Nature communications, 10(1), 1757.
279. Tien, J. F., Mazloomian, A., Cheng, S. G., Hughes, C. S., Chow, C., Canapi, L. T., Oloumi, A., Trigo-Gonzalez, G., Bashashati, A., Xu, J., Chang, V. C., Shah, S. P., Aparicio, S., & Morin, G. B. (2017). CDK12 regulates alternative last exon mRNA splicing and promotes breast cancer cell invasion. Nucleic acids research, 45(11), 6698–6716.
280. Schneeweiss, A., Park-Simon, T. W., Albanell, J., Lassen, U., Cortés, J., Dieras, V., May, M., Schindler, C., Marmé, F., Cejalvo, J. M., Martinez-Garcia, M., Gonzalez, I., Lopez-Martin, J., Welt, A., Levy, C., Joly, F., Michielin, F., Jacob, W., Adessi, C., Moisan, A., … Cervantes, A. (2018). Phase Ib study evaluating safety and clinical activity of the anti-HER3 antibody lumretuzumab combined with the anti-HER2 antibody pertuzumab and paclitaxel in HER3-positive, HER2-low metastatic breast cancer. Investigational new drugs, 36(5), 848–859.
281. Yaeger, R., Chatila, W. K., Lipsyc, M. D., Hechtman, J. F., Cercek, A., Sanchez-Vega, F., Jayakumaran, G., Middha, S., Zehir, A., Donoghue, M., You, D., Viale, A., Kemeny, N., Segal, N. H., Stadler, Z. K., Varghese, A. M., Kundra, R., Gao, J., Syed, A., Hyman, D. M., … Schultz, N. (2018). Clinical Sequencing Defines the Genomic Landscape of Metastatic Colorectal Cancer. Cancer cell, 33(1), 125–136.
282. Cancer Genome Atlas Network (2012). Comprehensive molecular characterization of human colon and rectal cancer. Nature, 487(7407), 330–337.
283. Duncan, R., Bazar, L., Michelotti, G., Tomonaga, T., Krutzsch, H., Avigan, M., & Levens, D. (1994). A sequence-specific, single-strand binding protein activates the far upstream element of c-myc and defines a new DNA-binding motif. Genes & development, 8(4), 465–480.

284. Hsiao, H. H., Nath, A., Lin, C. Y., Folta-Stogniew, E. J., Rhoades, E., & Braddock, D. T. (2010). Quantitative characterization of the interactions among c-myc transcriptional regulators FUSE, FBP, and FIR. Biochemistry, 49(22), 4620–4634.
285. Jacob, A. G., Singh, R. K., Mohammad, F., Bebee, T. W., & Chandler, D. S. (2014). The splicing factor FUBP1 is required for the efficient splicing of oncogene MDM2 pre-mRNA. The Journal of biological chemistry, 289(25), 17350–17364.
286. Lee, S. C., & Abdel-Wahab, O. (2016). Therapeutic targeting of splicing in cancer. Nature medicine, 22(9), 976–986.
287. Urbanski, L. M., Leclair, N., & Anczuków, O. (2018). Alternative-splicing defects in cancer: Splicing regulators and their downstream targets, guiding the way to novel cancer therapeutics. Wiley interdisciplinary reviews. RNA, 9(4), e1476.
288. Zhang, Y., Qian, J., Gu, C., & Yang, Y. (2021). Alternative splicing and cancer: a systematic review. Signal transduction and targeted therapy, 6(1), 78.
289. Cheung, L. W., Yu, S., Zhang, D., Li, J., Ng, P. K., Panupinthu, N., Mitra, S., Ju, Z., Yu, Q., Liang, H., Hawke, D. H., Lu, Y., Broaddus, R. R., & Mills, G. B. (2014). Naturally occurring neomorphic PIK3R1 mutations activate the MAPK pathway, dictating therapeutic response to MAPK pathway inhibitors. Cancer cell, 26(4), 479–494.
290. Elman, J. S., Ni, T. K., Mengwasser, K. E., Jin, D., Wronski, A., Elledge, S. J., & Kuperwasser, C. (2019). Identification of FUBP1 as a Long Tail Cancer Driver and Widespread Regulator of Tumor Suppressor and Oncogene Alternative Splicing. Cell reports, 28(13), 3435–3449.
291. Sailo, B. L., Banik, K., Girisa, S., Bordoloi, D., Fan, L., Halim, C. E., Wang, H., Kumar, A. P., Zheng, D., Mao, X., Sethi, G., & Kunnumakkara, A. B. (2019). FBXW7 in Cancer: What Has Been Unraveled Thus Far?. Cancers, 11(2), 246.
292. Wyatt, D. W., Feng, W., Conlin, M. P., Yousefzadeh, M. J., Roberts, S. A., Mieczkowski, P., Wood, R. D., Gupta, G. P., & Ramsden, D. A. (2016). Essential Roles for Polymerase θ-Mediated End Joining in the Repair of Chromosome Breaks. Molecular cell, 63(4), 662–673.
293. Feng, W., Simpson, D. A., Carvajal-Garcia, J., Price, B. A., Kumar, R. J., Mose, L. E., Wood, R. D., Rashid, N., Purvis, J. E., Parker, J. S., Ramsden, D. A., & Gupta, G. P. (2019). Genetic determinants of cellular addiction to DNA polymerase theta. Nature communications, 10(1), 4286.