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NEW PHARMACOLOGICAL APPROACHES IN THE TREATMENT OF ONCOLOGICAL DISEASES

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ABSTRACT

Malignant diseases are significant and growing health problem in almost all countries of the world. Besides the results achieved in reduction of morbidity and mortality in individual countries, significant progress of science and practice of oncology medicine and the application of new methods of diagnosis and treatment, cancer remains the second leading cause of death after cardiovascular diseases. The past decade has seen breakthroughs in personalized cancer medicine, where new targeted therapies are being developed which inhibit cellular proliferation and survival in tumors with certain specific oncogenic mutations. These new treatment approaches have shown progress in the understanding of the origin and pathogenesis of tumors, and offer hope for a good outcome of neoplastic diseases. In this review the idea is to present up-to-date report on these new molecular mechanisms and to identify their advantages and disadvantages from the pharmacological point of view.

Key words: antineoplastic drugs, oncology diseases, tyrosine-kinase inhibitors, cyclin-dependent kinases, monoclonal antibodies.

EXHIBITION

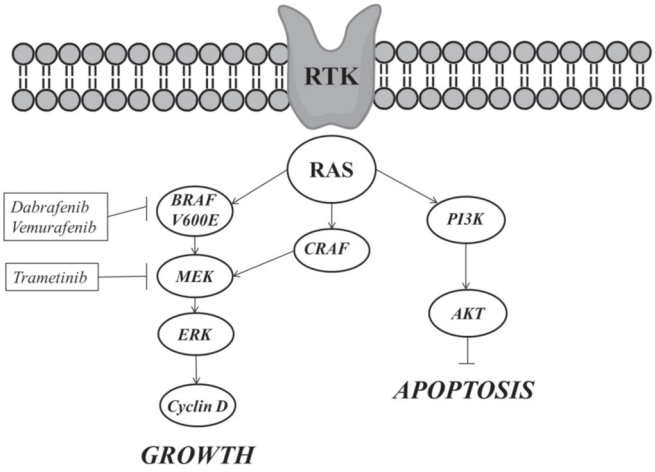
Melanoma is the deadliest form of skin cancer and has an incidence that is rising faster than any other solid tumor [1]. Currently, there are few effective treatments for disseminated melanoma and the median survival is 6–10 months. However, metastatic or unresectable melanoma treatment has improved considerably in the last five years since the introduction of the targeted therapy (BRAF and MEK inhibitors) and the immune checkpoint blockade (anti-CTLA4, anti-PD-1, and anti-PD-L1).

A significant advance in our understanding of melanoma initiation and progression was the discovery of activating mutations in BRAF. Approximately 40-60% of malignant melanomas have gene mutations at codon 600 of the BRAF gene (BRAF V600E, substitution of valine to glutamic acid) that result in constitutively activation of the mitogen-activated protein kinase (MAPK) pathway [2].

Targeting MEK

Trametinib (Mekinist®, GlaxoSmithKline) is the first-in-class ***mitogen-activated, extracellular signal-regulated kinase (MEK)***1 and 2 activation and kinase activity inhibitor that targets a kinase in the MAPK pathway that plays a key role in oncogenic cell proliferation, survival, invasion, tumor angiogenesis, and escape from apoptosis (fig.1) [3].

Figure 1. Cell signaling pathways activated in myeloma cells through receptor tyrosine kinases (RTK).



MEK is a downstream effector of the protein kinase B-raf (BRAF) and its inhibition through trametinib causes decreased cellular proliferation, cell cycle arrest, and increased apoptosis [4]. Trametinib is approved for treatment of unresectable or metastatic melanoma in patients with a BRAF V600E or BRAF V600K mutation (as detected by an approved test), either as a single-agent or in combination with dabrafenib. The combination, which targets two different tyrosine kinases in the RAS/RAF/MEK/ERK pathway, trametinib and dabrafenib (Tafinlar®, GlaxoSmithKline), RAF kinase inhibitor, allows greater inhibition of the MAPK pathway, resulting in BRAF V600 melanoma cell death [5]. The most common adverse effects of trametinib include rash, diarrhea, fatigue, peripheral edema, and nausea. The prevalence of severe adverse events is similar in combination therapy and in monotherapy [6].

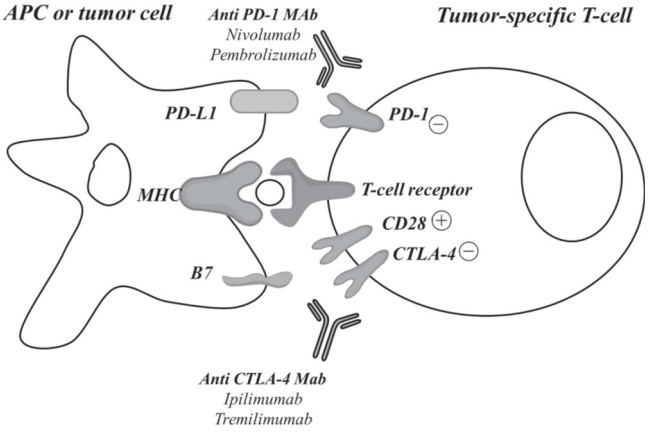
Targeting immune checkpoint blockade

Programmed cell death protein 1, also known as PD-1, is an immunoinhibitory receptor that belongs to the CD28 family and is expressed on T cells, B cells, monocytes, natural killer cells, and many tumor-infiltrating lymphocytes (TILs) [7]. PD-1 binds to 2 ligands that have been described – PD-L1 and PD-L2 [8]. Activation of this receptors leads to suppression of T-cell proliferation, cytokine production, and cell adhesion [9]. To avoid the destructive power of the

immune system, certain tumors upregulate expression of PD-1 ligands and this mark has been correlated with poor prognosis [10, 11]. Pharmacological approach in influencing this pathway, by which tumors escape immune response, can overcome resistance to tumors and help tumor-specific T cells to carry out their cytotoxic functions [12].

Nivolumab (Opdivo®,BMS-936558/MDX-1106, Bristol-Myers Squibb)is fully human immunoglobulin G4(IgG4) monoclonal antibody that selectively inhibits programmed cell death-1 (PD-1) activity by binding to the PD-1 receptor in order to block the ligands PD-L1 and PD-L2 and escape binding (fig.2).

Figure 2. Approaches of immunotherapy to restore the normal function of tumor specific T-cells.



The negative PD-1 receptor signaling that regulates T-cell activation and proliferation is therefore disrupted [13]. It was approved at the end of the previous year for treatment of patients with unresectable or advanced melanoma. In March this year, FDA approved the drug as the first immunotherapy for patients whose metastatic squamous non–small cell lung cancer (NSCLC) returns during or after treatment with platinum-based chemotherapy. The dosage which is used in both indications is 3 mg/kg intravenously once every 2 weeks until disease progression or unacceptable toxicity.The most common side effects are fatigue, shortness of breath, musculoskeletal pain, loss of appetite, cough, nausea and constipation.

Pembrolizumab (Keytruda®,formerly lambrolizumab, MK-3475,Merck) is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with ligands, PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the antitumor immune response. Blocking PD-1 activity is believed to prevent inhibition of T-cell immune surveillance of tumors and, in some models, has resulted in decreased tumor growth. It is approved for treatment of unresectable or metastatic melanoma with disease progression following ipilimumab (see below) and a BRAF inhibitor (if BRAF V600 mutation positive).

Other pharmacoimmunological approach is the blockade of cytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4(CD152) is upregulated early during the T-cell activation and its expression dampens T cells activation and proliferation by outcompeting CD28 in binding CD80 (B 7.1) and CD86 (B 7.2) (fig.2) [14, 15].

Ipilimumab (Yervoy®, Bristol-Myers Squibb, Princeton, NJ, USA)is a recombinant human IgG1 immunoglobulin monoclonal antibody. It was approved in March 2011 by FDA for treatment of unresectable or metastatic melanoma at a dose of 3 mg/kg i.v.every 3 weeks for 4 doses. Ipilimumab binds to the CTLA-4 with greater affinity than CD80 and CD86, inducing a cell-mediated immune response and breaking down T-cell tolerance to finally reduce tumor burden. It acts indirectly as enhancing of T-cell mediated immune response. In melanoma patients, the mean lymphocyte count increased in the peripheral blood throughout the induction phase depending on the dose [16]. The most common side effects are diarrhea, rash, itching, fatigue, nausea and vomiting, decreased appetite, and abdominal pain. These were mostly mild to moderate (grade 1 or 2). However, 10 to 15 percent of patients suffered severe immune-related adverse events (irAEs) of grade 3 or 4 and included diarrhea/colitis (8%), endocrinopathy (2%), dermatologic toxicity (< 2%), and hepatic toxicity (< 1%). Most irAEs resolved by 6 to 8 weeks with appropriate immunosuppressive treatment (mostly glucocorticoids), although residual symptoms (e.g., vitiligo, endocrinopathy symptoms, rectal pain) were sometimes present in long-term survivors [17].

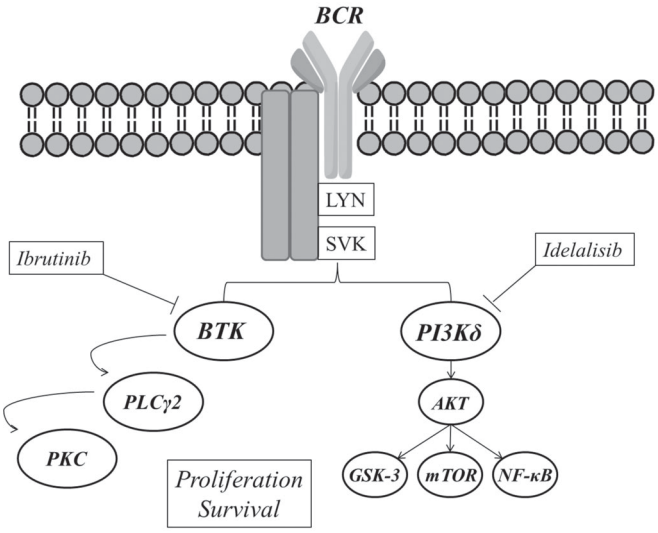
New therapies have helped to improve the outcome of the unresectable or metastatic melanoma, but not without some drawbacks, such as low-response rate (immune checkpoint blockade) or short-term responses (targeted therapy) [18]. However, combination therapies using this agent along with other targeted therapies and immunotherapies are currently under investigation.

Chronic lymphocytic leukemia (CLL) is one of the most common leukemias in the Western world, characterized by the monoclonal proliferation of mature B lymphocytes in the blood, lymph nodes, and marrow. The median age at diagnosis is around 70 years. For patients with CLL with 17p deletion or TP53 mutation previously there have been less promising treatment options available. Recently several new chemo-free treatment options have been introduced within clinical trials.

Targeting BTK

Ibrutinib (Imbruvica®, Janssen) is a potent and irreversible inhibitor of ***Bruton's tyrosine kinase (BTK)***, by covalently binding to a cysteine residue (Cys-481) in the active site of BTK. BTK is an integral component of the B-cell receptor (BCR) and cytokine receptor pathways and is important for survival of malignant B-cells (fig. 3).

Figure 3. Potential therapeutic targets for prevention of the B-cell receptor (BCR) activation.



The kinase inhibition with ibrutinib, this impedes the uncontrolled proliferation of malignant B-cell proliferation and survival. It is approved for treatment of patients with chronic lymphocytic leukemia (CLL, also with 17p deletion) and mantle cell lymphoma (MCL) who have received at least 1 prior therapy. Pharmacokinetic parameters of ibrutinib were rapid absorption, with bioavailability of 67% (in fasting state), volume of distribution ~ 10 000 L, oral plasma clearance ~ 1000 L/h and half-life elimination ~ 4 to 6 hours [19]. The dose used for the treatment of CLL, including 17p depletion was 420 mg p.o. once daily [20] and 560 mg p.o. once daily in MCL [21]. The most common adverse reactions in pivotal clinical trials were diarrhea, musculoskeletal pain, infections of the upper respiratory tract, bruises, rashes, nausea, fever, neutropenia, and constipation. The most common side effects of grade 3 and 4 were anemia, neutropenia, pneumonia and thrombocytopenia.

Targeting PI3Kä

Idelalisib (Zydelig®, GS-1101, Gilead Sciences) is a first-in-class, orally administered, ***phosphatidylinositol 3-kinase-ä*** inhibitor that was recently approved for the treatment of relapsed chronic lymphocytic leukemia (CLL) (in combination with rituximab) when rituximab alone is an appropriate therapy due to other comorbidities, relapsed follicular B-cell non-Hodgkin's lymphoma (NHL) and relapsed small lymphocytic lymphoma (SLL) in the USA and for the treatment of CLL and refractory follicular lymphoma in the EU. Idelalisib selectively inhibits the binding of adenosine-5'-triphosphate (ATP) to the catalytic domain of PI3Kä, whereby the phosphorylation of key lipid second messengers phosphatidylinositol and inhibited the phosphorylation of Akt (protein kinase B) are prevented. Idelalisib inhibits also several other signaling pathways, including B-cell, CXCR4 and CXCR5 receptor signaling, which may play important roles in CLL pathophysiology [22]. In addition, the new drug induces apoptosis and inhibits proliferation in cell lines derived from malignant B lymphocytes as well as in primary tumor cells. Most common side effects in the pivotal studies of idelalisib were infections, neutropenia, inflammatory changes in the lung, severe diarrhea, intestinal inflammation, rashes, fever, increased liver enzymes, and increased triglyceride levels. Possible interactions should be taken into account as the main metabolite – GS-563117is a potent inhibitor of CYP3A4 and drugs that are metabolized by this isoform should be avoided [23].

Targeting CD20

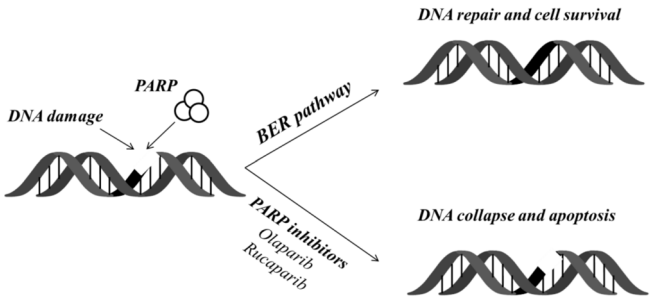
Ofatumumab (Arzerra®, GlaxoSmithKline) is a fully humanized monoclonal CD20 antibody approved for treatment of previously untreated CLL (in combination with chlorambucil) when fludarabine-based therapy is considered inappropriate and treatment of CLL refractory to fludarabine and alemtuzumab.It has a new mechanism of action, which is associated with the selective binding to the "large and small loop" of the CD20 molecule, thus enhances the action of the complement systemand antibody-dependent cell-mediated toxicity, which, in turn, to a greater extent destroys the malignant B-cells. The binding of the "small loop" thus differs from the binding site of the second currently available CD20 antibody rituximab. This explains the higher efficacy towards chronic lymphocytic leukemia (CLL) cells compared to rituximab [24]. In addition, ofatumumab remains longer at the binding place, resulting in a greater activation of the immune system – may be an indication for the particularly high effectiveness of the new drug, even at low levels of CD20 expression, as is the case in CLL. Ofatumumab was found to be well tolerated, some side effects that occurred in at least 10 percent of the patients included infections, anemia, neutropenia, and skin rashes.

The known under the name of BRCA 1 and 2 mutations can lead to breast and ovarian cancer in hereditary predisposed women. BRCA are tumor suppressor genes that are involved in DNA repair at the double strand, called homologous recombination. In addition, the known cell repair mechanism in the single-stranded is involved in the enzyme poly ADP-ribose polymerase (PARP).

Targeting PARP

Olaparib (Lynparza®,AZD-2281, AstraZeneca)is indicated for monotherapy in patients with deleterious or suspected deleterious germline BRCA-mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy.Olaparib is an inhibitor of ***poly (ADP-ribose) polymerase (PARP)*** enzymes, including PARP1, PARP2, and PARP3. PARP enzymes are involved in normal cellular homeostasis such as DNA transcription, cell cycle regulation, and DNA repair. The PARPs have a particularly critical role in the BER pathway, binding to single-strand breaks (SSBs) in DNA, modifying proteins in the vicinity, and ultimately leading to the recruitment of DNA repair proteins to the sites of damage (fig.4).

Figure 4. Function of PARP in DNA repair.



Cytotoxicity of olaparib may involve inhibition of PARP enzymatic activity, which induces synthetic lethality in BRCA1/2 deficient tumor cells through increased formation of PARP-DNA complex, resulting in disruption of cellular homeostasis and cell death [25]. The cells with functioning BRCA gene are not affected. Olaparib may be used as monotherapy for the treatment of adult patients with platinum-sensitive recurrence of a BRCA-mutated ovarian, fallopian tube or peritoneal cancer. It is available orally in capsule dosage form, which is administered 400 mg twice daily.Most common side effects are nausea, vomiting, fatigue and anemia.

Cellular proliferation, survival and growth are tightly controlled by the cell-cycle regulatory enzymes, serine/threonine kinases, which form complexes with cyclins. Cell cycle is composed of four phases – the S phase of DNA synthesis, the M phase of mitosis, and two gap phases, G1 and G2 [26]. There are four proliferative CDKs: CDK1 regulates the transition from G2 to M phase and CDK2, -4, and -6 regulate the transition from G1 to S phase [27]. However, CDK4 and -6 are critical drivers of oncogenesis in some tumors and therefore an attractive target for drug development [28]. Furthermore, CDK4/6 inhibitors are more efficient and less toxic antineoplastic agents than molecules targeting other CDKs [29].

Inhibition of cyclin-dependent kinases (CDKs)

Palbociclib (Ibrance®, PD-0332991,Pfizer) is a potent and selective inhibitor of CDK-4 and -6, which are critical components of the cell-cycle regulatory machinery. Palbociclib is approved by FDA in combination with letrozole, as a first-line treatment for postmenopausal women with estrogen receptor positive (ER+), human epidermal growth factor receptor 2 negative (HER2-) advanced breast cancer who have not received previous systemic treatment for their advanced disease. The accelerated submission is based on the final results of PALOMA-1, a randomized phase II study with 165 postmenopausal women with advanced or metastatic breast cancer with (ER +)/(HER2-) comparing palbociclib (125 mg once daily for three out of four weeks in repeated cycles) plus letrozole versus letrozole alone (2.5 mg once daily on a continuous regimen) in this population of patients. Combination therapy with palbociclib doubled disease-free survival (20.2 months versus 10.2 months) [30].

CONCLUSION

Over the past 50 years, the efficacy of cancer chemotherapy has improved considerably. However, the overall survival and other outcome parameters in metastatic cases have changed only modestly. More key molecules have been identified as targets for specific drugs due to the advanced knowledge of molecular pathways and regulatory processes within tumor cells. These could improve established therapeutic strategies, such as humanized antibodies or small-molecule inhibitors directed against growth regulatory kinases, or brand new approaches. Significant progress in this area gives hope for individualized therapy with detection of specific gene mutations, which could contribute to better outcome of neoplastic diseases. However, it takes time of these new approaches to be confirmed in clinical practice, but there is light in the tunnel.

REFERENCES:

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer Statistics, 2014. *CA Cancer J Clin.* 2014 Jan-Feb;64(1):9-29. [CrossRef]

2. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. *Nature.* 2002 Jun 27; 417 (6892):949-54. [PubMed] [CrossRef]

3. Chung C, Reilly S. Trametinib: A novel signal transduction inhibitor for the treatment of metastatic cutaneous melanoma. *Am J Health Syst Pharm.* 2015 Jan 15;72(2):101-110. [PubMed] [CrossRef]

4. Kim KB, Kefford R, Pavlick AC, Infante JR, Ribas A, Sosman JA, et al. Phase II study of the MED1/MEK2 Inhibitor Trametinib In Patients With Metastatic BRAF-Mutant Cutaneous Melanoma Previously Treated With Or Without A BRAF Inhibitor. *J Clin Oncol.* 2013 Feb;31(4):482-9. [PubMed] [CrossRef]

5. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J et al. Combined BRAF and MEK Inhibition in Melanoma With BRAF V600 Mutations. *N Engl J Med.* 2012 Nov; 367 (18):1694-703. [PubMed] [CrossRef]

6. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N Engl J Med.* 2015 Jan;372(1):30-9. [PubMed] [CrossRef]

7. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol.* 2008; 26:677-704. [PubMed] [CrossRef]

8. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol.* 2001 Mar;2(3):261-268. [PubMed] [CrossRef]

9. Ghiotto M, Gauthier L, Serriari N, Pastor S, Truneh A, Nunès JA, et al. PD-L1 and PD-L2 differ in their molecular

mechanisms of interaction with PD-1. *Int Immunol.* 2010 Aug;22(8):651-660. [PubMed] [CrossRef]

10. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature.* 2011 Dec:480(7378);480-489. [PubMed] [CrossRef]

11. Zitvogel L, Kroemer G. Targeting PD-1/PD-L1 interactions for cancer immunotherapy. *Oncoimmunology.* 2012 Nov;1(8):1223-1225. [PubMed] [CrossRef]

12. Tang PA, Heng DY. Programmed death 1 pathway inhibition in metastatic renal cell cancer and prostate cancer. *Curr Oncol Rep*. 2013 Apr;15(2): 98-104. [PubMed] [CrossRef]

13. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med.* 2015 Jan 22;372(4):320-30. [PubMed] [CrossRef].

14. Egen JG, Allison JP. Cytotoxic T lymphocyte antigen-4 accumulation in the immunological synapse is regulated by TCR signal strength. *Immunity*. 2002 Jan;16(1):23–35. [PubMed] [CrossRef]

15. Riley JL, Mao M, Kobayashi S, Biery M, Burchard J, Cavet G, et al Modulation of TCR-induced transcriptional profiles by ligation of CD28, ICOS, and CTLA-4 receptors. *Proc Natl Acad Sci USA*. 2002 Sep;99(18): 11790-11795. [PubMed] [CrossRef]

16. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010 Aug; 363(8):711-23. [PubMed] [CrossRef]

17. Camacho LH. CTLA-4 blockade with ipilimumab: biology, safety, efficacy, and future considerations. *Cancer Med.* 2015 May;4(5):661-672. [PubMed] [CrossRef]

18. Kim T, Amaria RN, Spencer C, Reuben A, Cooper ZA, Wargo JA. Combining targeted therapy and immune checkpoint inhibitors in the treatment of metastatic melanoma. *Cancer Biol Med.* 2014 Dec;11(4):237-46. [PubMed] [CrossRef].

19. Marostica E, Sukbuntherng J, Loury D, de Jong J, de Trixhe XW, Vermeulen A, et al. Population pharmacokinetic model of ibrutinib, a Bruton tyrosine kinase inhibitor, in patients with B cell malignancies. *Cancer Chemother Pharmacol*. 2015 Jan; 75(1):111-21. [PubMed] [CrossRef].

20. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2013 Jul 4;369(1):32-42. [PubMed] [CrossRef]

21. Wang ML, Rule S, Martin P, Goy A, Auer R, Kahl BS, et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *N Engl J Med.* 2013 Aug 8;369(6):507-16. [PubMed] [CrossRef]

22. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2014 Mar 13;370(11):997-1007. [CrossRef]

23. Liewer S, Huddleston AN. Oral targeted therapies: managing drug interactions, enhancing adherence and optimizing medication safety in lymphoma patients. *Expert Rev Anticancer Ther.* 2015 Apr;15(4):453-64. [CrossRef]

24. Grosicki S. Ofatumumab for the treatment of chronic lymphocytic leukemia. *Expert Rev Hematol.* 2015 8(3):265-72. [PubMed] [CrossRef]

25. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med.* 2012 Apr 12; 366 (15):1382-92. [CrossRef]

26. Weinberg RA. The Biology of Cancer. 2nd ed. New York: Garland Science; 2014.

27. Roberts PJ, Bisi JE, Strum JC, Combest AJ, Darr DB, Usary JE, et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. *J Natl Cancer Inst.* 2012 Mar;104(6):476-487. [PubMed] [CrossRef]

28. Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. *Mol Cancer Ther.* 2004 Nov;3(11):1427-1438. [PubMed]

29. Roberts PJ, Bisi JE, Strum JC, Combest AJ, Darr DB, Usary JE, et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. *J Natl Cancer Inst.* 2012 Mar; 104(6): 476-487. [PubMed] [CrossRef]

30. Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. *Lancet Oncol*. 2015 Jan;16(1):25-35. [PubMed] [CrossRef]

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