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Targeted cancer therapy – Are the days of systemic chemotherapy numbered?

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Abstract

Targeted therapy or molecular targeted therapy has been defined as a type of treatment that blocks the growth of cancer cells by interfering with specific cell molecules required for carcinogenesis and tumor, rather than by simply interfering with all rapidly dividing cells as with traditional chemotherapy. There is a growing number of FDA approved monoclonal antibodies and small molecules targeting specific types of cancer suggestive of the growing relevance of this therapeutic approach. Targeted cancer therapies, also referred to as “Personalized Medicine”, are being studied for use alone, in combination with other targeted therapies, and in combination with chemotherapy. The objective of personalized medicine is the identification of patients that would benefit from a specific treatment based on the expression of molecular markers. Examples of this approach include bevacizumab and olaparib, which have been designated as promising targeted therapies for ovarian cancer. Combinations of trastuzumab with pertuzumab or T-DM1 and mTOR inhibitors added to an aromatase inhibitor are new therapeutic strategies for breast cancer. Although this approach has been seen as a major step in the expansion of personalized medicine, it has substantial limitations including its high cost and the presence of serious adverse effects. The Cancer Genome Atlas is a useful resource to identify novel and more effective targets, which may help to overcome the present limitations. In this review we will discuss the clinical outcome of some of these new therapies with a focus on ovarian and breast cancer. We will also discuss novel concepts in targeted therapy, the target of cancer stem cells.

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Contributors

The authors did the literature review and preparation of the manuscript. WDJ and GM planned and constructed the structure of the manuscript, searched and reviewed literature, and wrote the manuscript. IV reviewed and finalized the manuscript.

Competing interest

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Keywords

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1. History of targeted cancer therapy

Targeted cancer therapy has attracted public attention with the hope that it will be possible to replace systemic chemotherapy in the future. This ‘magic bullet’ therapy is expected to be more effective and less harmful than systemic chemotherapy because the aim of targeted cancer therapy is to block specific pathways related to carcinogenesis and tumor growth by inducing apoptosis of cancer cells, blocking specific enzymes and growth factor receptors involved in cancer cell proliferation, or modifying the function of proteins that regulate gene expression and other cellular functions, rather than by simply interfering with all rapidly growing cells. If it is possible, the goal of cancer treatment in the future will be shifted from ‘cure’ to ‘management’ and cancer patients will not be expected to experience hair loss, which is still a stereotype of systemic chemotherapy.

Surprisingly, this concept is nothing new and it has been available for a long time. A classical model of targeted cancer therapy is ^{131}I therapy for thyroid cancer. Thyroid cancer cells exclusively uptake iodine by its iodine receptor and the accumulated radioactivity of ^{131}I kills thyroid cancer cells.[1] This targeted therapy for thyroid cancer has been used successfully since the 1940s.[2] A more typical model of molecular targeted therapy is tamoxifen, a selective estrogen receptor modulator (SERM). It binds to estrogen receptors competitively and antagonizes them in breast tissue. Because some breast cancer cells require estrogen to grow, tamoxifen has been used to prevent recurrence of estrogen receptor-positive breast cancer for pre- and post-menopausal women.[3]

One of the first breakthrough of molecular target biology was imatinib, used for the treatment of chronic myeloid leukemia (CML). Philadelphia chromosome, a unique characteristic of CML, is related to BCR-Abl tyrosine kinase overexpression, which does not occur in normal cells. Therefore, this selective BCR-Abl tyrosine kinase inhibitor, imatinib, could suppress the growth of Philadelphia chromosome-positive CML with less harm to normal cells.[4] Thereafter, CML seemed to become a ‘manageable’ disease, like hypertension or diabetes. Imatinib was also found to be effective in gastrointestinal stromal tumor (GIST) with c-kit overexpression.[5]

Due to the success of targeted cancer therapy in CML, a number of new drugs were developed for the treatment of solid tumors. Unfortunately, not all these new drugs were found to be effective in the majority of the tested tumor types. Gefitinib, an EGFR inhibitor, is an example of a new therapy that the U.S. Food and Drug Administration (FDA) initially approved for the treatment of non-small cell lung cancer (NSCLC). Two years later, the FDA withdrew the approval of gefitinib due to lack of evidence that it improved survival of patients.[6] The FDA also removed bevacizumab, a monoclonal antibody that inhibits angiogenesis, because of its lack of efficacy in breast cancer patients and its numerous side

effects.[7] In spite of these early disappointments, new-targeted cancer therapies are still under active investigation.

2. Categories of targeted therapies

Two categories of targeted cancer therapy include small molecules and monoclonal antibodies. Small molecules are referred to low molecular (less than 800 Dalton) organic compounds. These ‘small molecules’ can penetrate the cell membrane and are designed to interfere with signaling pathways and to act on targets found inside the cell. Most monoclonal antibodies cannot penetrate the cell’s plasma membrane and are designed against targets outside the cell or on the cell surface. The name of a targeted therapy provides a clue to the type of agent and its cellular target. Small molecules that have “-ib” as a suffix indicate a molecule that has inhibitory properties. Many of these molecules are developed as tyrosine kinase inhibitors. Imatinib and gefitinib, mentioned above, are typical examples of small molecules with inhibitory potential. Erlotinib is an EGFR tyrosine kinase inhibitor and works similarly to gefitinib. Recently it was shown in the SATURN (Sequential Tarceva in Unresectable NSCLC) study that erlotinib was significantly better than gefitinib as maintenance treatment for advanced NSCLC.[8] Therefore erlotinib has replaced gefitinib for advanced NSCLC.

“Monoclonal antibodies” are designated humanized antibodies, which bind to cancer cell-specific antigens. Monoclonal antibodies have “-mab” as a suffix. The FDA approved four kinds of monoclonal antibodies for the treatment of solid tumors: bevacizumab, cetuximab, panitumumab, and trastuzumab. Bevacizumab targets vascular endothelial growth factor (VEGF). It is approved for colorectal cancer, non-small cell lung cancer (NSCLC), metastatic renal cancer and glioblastoma multiforme. Trastuzumab targets HER2/neu receptor and is used for HER2-positive metastatic breast cancer. Cetuximab targets epidermal growth factor receptor (EGFR) and is used for colorectal cancer and NSCLC. Rituximan targets CD20 on B cells and is used for non-Hodgkin’s lymphoma. Another therapeutic application for these monoclonal antibodies is their use as drug delivery system or antibody-drug conjugate (ADCs). When a monoclonal antibody binds to cancer cells, the cytotoxic drug conjugated with the antibody is engulfed into the cancer cells, released intracellular inducing specific cell death. This technology provides a wider therapeutic range by targeting cancer cells by reducing the potential side effects of the cytotoxic compound. The pioneer of ADCs was gemtuzumab-ozogamicin, approved for acute myeloid leukemia (AML) in 2001. But it was withdrawn from the market at the request of the FDA in 2010.[9, 10] In 2011, the FDA approved brentuximab-vedotin for relapse and refractory Hodgkin lymphoma and anaplastic large cell lymphoma.[11] In 2013, Ado-trastuzumab emtansine (T-DM1), which is trastuzumab linked to DM1, was approved by the FDA for HER2-positive metastatic breast cancer.[12]

3. Personalized medicine and The Cancer Genome Atlas (TCGA)

The successes and failures of these small molecules and antibodies further demonstrate the complexity of the tumor biology and the need to identify new specific pathways. Clearly, it will be very difficult to have a single therapy for all cancers, not even for a single type of

cancer. Therefore, the concept of personalized medicine becomes relevant and points to the need to evaluate every patient according to his/her unique tumor phenotype. Consequently, the next step of targeted cancer therapy is the identification of new specific targets. The identified target molecules will then be used for the identification of the specific sub-population of patients who have the receptor of the identified target molecule and therefore could benefit from the treatment. This is the major aim of “Personalized medicine”. Future FDA approval of a targeted therapy will be based on the identification of new drugs for a specific population of patients who have a specific marker. An example of this approach is trastuzumab, a monoclonal antibody that interferes with the HER2/neu receptor. An HER2 assay is required to administer trastuzumab because only patients that test positive for HER2-metastatic breast cancer have FDA approval to receive the drug.

Next generation sequencing (NGS) made whole-genome sequencing of cancer samples become a reality and enabled comprehensive research of cancers’ genome.[13] One of the biggest studies using this approach was The Cancer Genome Atlas (TCGA) project, whose aim was to reveal molecular aberrations that are critical for carcinogenesis and tumor growth in a variety of cancers, including ovarian cancer. The TCGA project analyzed mRNA expression, microRNA expression, promoter methylation and DNA copy number in 489 high-grade serous ovarian adenocarcinomas and the DNA sequences of exons from coding genes in 316 of these tumors. TP53 mutations were reported in 96% of tumors. Somatic mutations in 9 other genes, including NF1, BRCA1, BRCA2, RB1 and CDK12 were statically recurrent. Focal DNA copy number aberrations in 113 genes and promoter methylation in 168 genes were reported. BRCA1, BRCA2 and CCNE1 aberrations were associated with survival. NOTCH and FOXM1 signaling are involved in serous ovarian cancer pathophysiology. [14] This analysis provides a great opportunity to identify new targets for drug development, which can be applied in individual patients based on their tumor phenotype.

4. Clinical trials of targeted therapies for women’s cancer

For more than 40 years, since the introduction of platinum treatment, no other_comparable therapeutic modalities have been available for the treatment of gynecologic cancers, especially ovarian cancer. However, there are major efforts to introduce target therapy either alone or in combination with other treatments. Below are examples of currently approved targeted therapies for solid malignancies and their molecular targets for ovarian and breast cancers.

4.1 Bevacizumab in ovarian cancer

Two large randomized clinical trials using bevacizumab with chemotherapy, have been conducted in newly diagnosed patients as part of the Gynecologic Oncology Group (GOG) 218 and ICON7. GOG 218 reported a significant improvement in progression-free_survival (PFS) (14.1 vs. 10.3 months) in the patients who received bevacizumab with chemotherapy and maintenance.[15] ICON7 also showed a significant improvement in PFS (24 vs. 22 months) in bevacizumab arm.[16] The Ovarian Cancer Study Comparing Efficacy and Safety of Chemotherapy and Anti-angiogenic Therapy in Platinum-Sensitive Recurrent Disease (OCEANS) trial reported that additional bevacizumab with maintenance extended

PFS of 4 months (12.4 vs. 8.4 months) in platinum-sensitive recurrent disease.[17] However, overall survival (OS) was not improved in all three studies.[15–17]

4.2 PARP inhibitor in ovarian cancer

The integrated genome analyses of serous ovarian adenocarcinoma in TCGA database noted that about 50% of serous ovarian carcinoma might have disruption of the homologous recombination pathway and thus might be susceptible to Poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitors. PARP repairs single-strand DNA breaks, consequently, inhibition of PARP could enhance the cytotoxic effect of chemotherapy. Olaparib, an oral PARP inhibitor, was expected to show antitumor activity in patients with germline BRCA mutations. Unfortunately, an initial Phase II trial comparing olaparib and pegylated liposomal doxorubicin in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer failed to show PFS improvement.[18] However, a second phase II study of olaparib demonstrated significantly prolonged PFS (8.4 vs. 4.8 months) in patients with platinum-sensitive recurrent high-grade serous ovarian cancer regardless of BRCA1 or BRCA2 germline mutations. [19] These results suggest that there may be a group of patients who could benefit from this treatment; the challenge will be to identify a marker that can be use in the selection of those patients.

4.3 New targeted therapies in breast cancer

Pre-clinical studies have shown that the combination of trastuzumab and pertuzumab strongly enhanced antitumor activity than either agent alone in a HER2-positive human xenograft tumor model.[20] Pertuzumab, another anti-HER2 humanized monoclonal antibody, binds at a different epitope of HER2 extracellular domain from that at which trastuzumab binds. Therefore, combination of trastuzumab and pertuzumab gives more comprehensive blockade of HER2 signaling.[21] The Clinical Evaluation of the pertuzumab and trastuzumab (CLEOPATRA) study demonstrated significantly prolonged PFS (18.5 vs. 12.4 months) of pertuzumab plus trastuzumab plus docetaxel (pertuzumab group) compared to placebo plus trastuzumab plus docetaxel (control group) as first-line treatment for patients with HER2-positive metastatic breast cancer with no increase in cardiac toxicity. [22]

T-DM1, as mentioned above, is also a good candidate for the treatment of HER2-positive breast cancer. EMILIA study randomly assigned 991 patients with HER2-positive advanced breast cancer, previously treated with trastuzumab and a taxane, to T-DM1 or lapatinib plus capecitabine. Significantly prolonged PFS (9.6 vs. 6.4 months) as well as OS (30.9 vs. 25.1 months) were observed in T-DM1 group with less toxicity than lapatinib plus capecitabine group. [23]

Resistance to hormone therapy of breast cancer is associated with mTOR signaling pathway activation.[24] Everolimus, an oral mTOR inhibitor added to aromatase inhibitor showed antitumor activity.[25] The Breast Cancer Trial of Oral Everolimus-2 (BOLERO-2) study compared everolimus and exemestane versus exemestane and placebo in patients with hormone-receptor-positive advanced breast cancer. The interim analysis after 359 DFS events showed prolonged PFS in everolimus group (10.6 and 4.1 months). [26] Another mTOR inhibitor, temsirolimus, added to letrozole also showed promising results.

Randomized phase III placebo-controlled trial of letrozole plus oral temsirolimus as first-line endocrine therapy in postmenopausal women with locally advanced or metastatic breast cancer demonstrated improved PFS favoring letrozole/temsirolimus in patients under age 65 years (9.0 v 5.6 months) at the interim analysis after 382 PFS events.[27]

5. Future directions and obstacles to overcome

5.1 Tumor heterogeneity

The majority of the studies described above, have shown an effect on prolonging patient survival by months but have failed to cure the disease or transform it to a chronic condition. These results further challenge our basic understanding of the biology of cancer. The classical definition of malignant tumors is a mixture of fast dividing malignant cancer cells and non-malignant stromal cells. Mutation of cells gives rise to cancer clones that expand and form the bulk of the tumor. Recent studies have shown that tumors are not homogeneous and there is a wide range of intratumoral heterogeneity[13]. Additional mutations of cancer cells as they expand may give origin to additional subclones that have different genetic and molecular characteristics. As a consequence, the tumors from different patients have differences in genetic and molecular characteristics. Even the tumors from a single patient may have genetic and molecular diversities as well. The former is referred to as 'intertumoral' heterogeneity and the latter is referred to as 'intratumoral' heterogeneity. [13] Tumor heterogeneity has been reported in breast cancers, gliomas, bone and soft-tissue sarcomas, pancreatic cancers, and clear-cell renal cell carcinoma.[28–32] Such heterogeneities may confer the tumors heightened growth advantage or potential to seed metastases. In addition, DNA and RNA isolated from a bulk tumor are mixtures of malignant and non-malignant DNA and RNA. Biopsy samples from small tumor regions may not adequately represent entire cellular architecture and biomarker of the bulk tumor. [32].

The presence of tumor heterogeneity is a major challenge in developing effective targeted therapies for the treatment of solid tumors. Another explanation for tumor heterogeneity is the existence of a cell hierarchy within the cancer cells. The tumor may harbor a side population that is responsible for tumor initiation as well as for the resistance to chemotherapy and consequently it is the source of recurrence. When the main population of the tumor is destroyed by systemic chemotherapy, the side population may survive. This side population has been referred as “tumor initiating cells” or “cancer stem cells”. Therefore, to identify this side population and characterize its biomarkers will be the first step to develop a brand-new targeted therapy. In the aspect of personalized medicine, tumor heterogeneity of each patient should be taken into consideration if the genomic context of a tumor is to be used to guide therapy. The targeted therapies may be effective only when they are accompanied by a comprehensive understanding of the clonal and genetic architecture of the entire tumor and the underlying genetic characteristics of the side population constituting the tumor.[13]

5.2 Cancer stem cells (CSCs)

CSCs are undifferentiated, slow growing cells and have ability of self-renewal and differentiation into fast growing cancer cells. CSCs also have different gene expression profiles and can evade targeted cancer therapy for differentiated cancer cells. Hence, they survive and replenish after chemotherapy.[33] Several molecular markers for CSCs have been reported in different types of gynecologic tumors. CD44⁺/CD24^(-low), CD133⁺ for breast cancer[34, 35], CD44⁺/CD117⁻, CD44⁺/MyD88⁺, CD133⁺ and ALDH⁺ for ovarian cancer[36–39], and CD133⁺ for endometrial cancer[40].

CSCs are resistant to traditional chemotherapies compared with the rest of the cancer cells within the tumor mass. Why are CSCs resistant to current therapy? One of the answers is the presence of efflux mechanisms, including ATP binding cassette (ABC) and multi-drug resistance (MDR) transporter that remove cytotoxic chemicals from the cells.[41] For example, multidrug transporter protein BCRP1 was insensitive to doxorubicin, suggesting a possible link between CSCs and chemoresistance.[42] Another answer is heightened activation of DNA repair mechanisms, which enable enhanced radiation resistance or increased defenses against reactive oxygen species.[43] A more simplistic explanation is their slow growing characteristic that prevents many of the effects of conventional chemotherapy.

We propose a model of chemoresistance and targeted therapy against CSCs in figure 1. Strategies to target CSCs are (1) neutralizing the advantages of CSC to survive chemotherapy, (2) interrupting key survival pathways, and (3) targeting the CSC niche.[44] In terms of neutralizing the chemotherapy advantages of CSCs, ABC transporters (ABCC1 and ABCG2) inhibitors have been suggested as candidates for targeting CSCs and they might render the CSCs more sensitive to chemotherapy. However, the toxicity of ABC transporters is a major limiting factor. ABC transporters play a critical role in maintaining blood-brain barrier (BBB), hence they may also cause harm by inhibiting the maintenance of BBB or by rendering normal stem cells more vulnerable to chemotherapy.[45]

Another strategy to inhibit the growth of CSCs is interrupting key survival pathways. The TGF- β pathway has been associated with tumor growth, as well as with regression of differentiated cells into progenitor and stem cell lineages. Chemotherapy-induced TGF- β overexpression enhances tumor recurrence through IL-8-dependent expansion of CSCs and TGF- β pathway inhibitors prevent the development of drug-resistant CSCs.[46] Bone morphogenic protein (BMP) is a well-studied TGF- β inhibitor and it can inhibit tumor growth by differentiating tumor-initiating brain cells.[47] The Notch pathway is also correlated to cancer survival and proliferation, especially in some brain tumors. Inhibition of Notch pathway in medulloblastomas was shown to interrupt proliferation of tumor cells expressing CD133 both in vitro and in vivo.[48] Aberrant expression of the Sonic Hedgehog-homolog (SHH) pathway has also been implicated in the development of many kinds of cancers and a small molecule inhibitor of SHH, GDC-0449, has shown clinical effect on CSCs of lung cancer and pancreatic cancer. [49, 50] WNT pathway is also upregulated in a wide variety of cancers and Chimeric 5/35 adenovirus-mediated Dickkopf-1 (DKK1), a biological WNT blocker, overexpression suppressed tumorigenicity of CD44⁺ gastric cancer cells via attenuating WNT signaling.[51] Inhibition of Aurora-A kinase was

shown to induce cell cycle arrest in epithelial ovarian cancer stem cells by affecting the NF- κ B pathway. [52] In ovarian cancer stem cells, the NF- κ B pathway may represent a potential target since inhibition of NF- κ B was shown to enhance apoptosis [53] or to prevent differentiation into endothelial cells. [54] Inhibition of anti-apoptotic genes such as X-linked inhibitor of Apoptosis (XIAP) or Akt has been shown to be effective in inducing apoptosis in cancer stem cells. [55]

Although interrupting these key pathways have shown to be effective in inhibiting CSCs, once applied to patients may prove to be of limited efficacy due to the complexity of pathway interaction. Another issue is toxicity because many of these pathways are critical to the function and survival of normal stem cells. A new drug targeted to CSCs, such as salinomycin, is investigated to solve these problems.[56, 57]

The CSCs niche is believed to play important role on CSCs self-renewal allowing it to maintain the pool of CSCs; therefore targeting the CSCs niche could be an ideal approach for preventing the CSCs expansion.

5.3 Epithelial-mesenchymal transition

Epithelial-mesenchymal transition (EMT) is a process linked to the generation of cancer stem cells with metastatic potential and chemoresistance.[58, 59] Like Dr. Jekyll and Mr. Hyde, cancer cells are able to change their character. When cancer cells form a solid tumor, they are differentiated and maintain an epithelial phenotype. Epithelial cells have tight junctions and lack migration capability, therefore they cannot metastasize. In order to migrate, epithelial cells need to acquire mesenchymal properties, which include change in their morphology (spindle-shaped) and migratory capability. New studies from our laboratory suggest that chemotherapy may induce modifications associated with EMT in the surviving cells, which confers stemness and migratory properties.[60] Therefore, EMT is closely related to recurrence and metastasis and blocking EMT process may provide a major source for the development of target therapies to prevent recurrence and metastasis.

Transforming growth factor beta (TGF- β) induces EMT process, leading to cancer progression.[61] At the late stage of malignancy, tumor microenvironment is a target of TGF- β action that stimulates tumor progression via pro-tumor effects on vascular, immune and fibroblastic cells.[62] Therefore, TGF- β is a good candidate for targeted cancer therapy. TGF- β inhibitors have four categories; ligand trap (e.g. GC-1008), antisense oligonucleotide (e.g. AP12009), receptor kinase inhibitor (e.g. LY2157299) and peptide aptamer. Those drugs were tested in Phase I/II trials for safety and efficacy.[62–64] Clinically TGF- β inhibitors have shown less robust effects than hoped for, while metastasis in mouse models was significantly decreased, which suggest that combinatorial therapy may increase the efficacy of TGF- β inhibitors rather than TGF- β inhibitors alone in a clinical setting.[62]

Knockdown of the TGF- β signaling pathway has been known to enhance the therapeutic efficacy of cytotoxic agents such as rapamycin and doxorubicin.[65, 66] Moreover, treatment of triple-negative breast cancer xenografts with the TGF- β type I receptor kinase inhibitor LY2157299 prevented re-establishment of tumors after paclitaxel treatment. These findings support testing a combination of TGF- β inhibitors and anticancer chemotherapy.

[46] A clinical trial evaluating the effects the T β RI kinase inhibitor LY2157299 in combination with gemcitabine (nucleoside analog) in metastatic and advanced unresectable pancreatic cancer is currently recruiting participants (clinical trial ID NCT01373164).

6. Conclusion

Though some of the available targeted cancer therapies have significantly improved PFS, none of these therapies have yet proved to cure the disease. Furthermore, for those therapies that have shown some clinical benefits, high cost remains a major obstacle for its implementation. Nevertheless, targeted cancer therapy is still our hope to achieve the objective of making cancer a chronic disease as well as to decrease the side effects associated with chemotherapy. Recognition of tumor heterogeneity and the presence of multiple types of cancer cells hierarchically organized within the tumor, suggest that the future of personalized medicine will imply not only to target a specific cellular pathway within the tumor but a molecular target within a specific cell type which can be defined as cell-type-based therapy.

References

1. Weigel RJ, McDougall IR. The role of radioactive iodine in the treatment of well-differentiated thyroid cancer. *Surg Oncol Clin N Am*. 2006; 15:625–638. [PubMed: 16882501]
2. Lee SL. Radioactive iodine therapy. *Curr Opin Endocrinol Diabetes Obes*. 2012; 19:420–428. [PubMed: 22914564]
3. Jordan VC. Fourteenth Gaddum Memorial Lecture. A current view of tamoxifen for the treatment and prevention of breast cancer. *Br J Pharmacol*. 1993; 110:507–517. [PubMed: 8242225]
4. Fausel C. Targeted chronic myeloid leukemia therapy: Seeking a cure. *Am J Health Syst Pharm*. 2007; 64:S9–S15. [PubMed: 18056932]
5. Dematteo RP, Ballman KV, Antonescu CR, Maki RG, Pisters PW, Demetri GD, et al. American College of Surgeons Oncology Group Intergroup Adjuvant GST. Adjuvant imatinib mesylate after resection of localised, primary gastrointestinal stromal tumour: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2009; 373:1097–1104. [PubMed: 19303137]
6. FDA. Gefitinib (marketed as Iressa) information. FDA ALERT; p. Postmarket Drug Safety Information for Patients and Providers. [6/2005]
7. Pollack A. FDA rejects use of drug in cases of breast cancer. *NY Times*. 2010
8. Cappuzzo F, Ciuleanu T, Stelmakh L, Cicen S, Szczesna A, Juhasz E, et al. SATURN investigators. Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. *Lancet Oncol*. 2010; 11:521–529. [PubMed: 20493771]
9. FDA. Mylotarg (gemtuzumab ozogamicin): Market withdrawal. FDA Safety Alerts for Human Medical Products. 2010
10. Richwine, L. Pfizer pulls leukemia drug from U.S. market. *Reuters*; 2010.
11. GEN News. Seattle Genetics' antibody-drug conjugate receives FDA okay to treat lymphomas. *GEN News*; 2011.
12. Neale T. FDA OKs Genentech breast cancer drug. *Medpage Today*. 2013
13. Samuel N, Hudson TJ. Translating genomics to the clinic: implications of cancer heterogeneity. *Clin Chem*. 2013; 59:127–137. [PubMed: 23151419]
14. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011; 474:609–615. [PubMed: 21720365]
15. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Gynecologic Oncology Group. Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N Engl J Med*. 2011; 365:2473–2483. [PubMed: 22204724]

16. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al. ICON Investigators. A phase 3 trial of bevacizumab in ovarian cancer. *N Engl J Med*. 2011; 365:2484–2496. [PubMed: 22204725]
17. Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. *J Clin Oncol*. 2012; 30:2039–2045. [PubMed: 22529265]
18. Kaye SB, Lubinski J, Matulonis U, Ang JE, Gourley C, Karlan BY, et al. Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer. *J Clin Oncol*. 2012; 30:372–379. [PubMed: 22203755]
19. 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; 366:1382–1392. [PubMed: 22452356]
20. Scheuer W, Friess T, Burtscher H, Bossenmaier B, Endl J, Hasmann M. Strongly enhanced antitumor activity of trastuzumab and pertuzumab combination treatment on HER2-positive human xenograft tumor models. *Cancer Res*. 2009; 69:9330–9336. [PubMed: 19934333]
21. Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell*. 2004; 5:317–328. [PubMed: 15093539]
22. Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, et al. CLEOPATRA Study Group. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med*. 2012; 366:109–119. [PubMed: 22149875]
23. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med*. 2012; 367:1783–1791. [PubMed: 23020162]
24. Johnston SR. Clinical efforts to combine endocrine agents with targeted therapies against epidermal growth factor receptor/human epidermal growth factor receptor 2 and mammalian target of rapamycin in breast cancer. *Clin Cancer Res*. 2006; 12:1061s–1068s. [PubMed: 16467125]
25. Baselga J, Semiglazov V, van Dam P, Manikhas A, Bellet M, Mayordomo J, et al. Phase II randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor-positive breast cancer. *J Clin Oncol*. 2009; 27:2630–2637. [PubMed: 19380449]
26. Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med*. 2012; 366:520–529. [PubMed: 22149876]
27. Wolff AC, Lazar AA, Bondarenko I, Garin AM, Brincaat S, Chow L, et al. Randomized phase III placebo-controlled trial of letrozole plus oral temsirolimus as first-line endocrine therapy in postmenopausal women with locally advanced or metastatic breast cancer. *J Clin Oncol*. 2013; 31:195–202. [PubMed: 23233719]
28. Gray JW, Collins C, Henderson IC, Isola J, Kallioniemi A, Kallioniemi OP, et al. Molecular cytogenetics of human breast cancer. *Cold Spring Harb Symp Quant Biol*. 1994; 59:645–652. [PubMed: 7587125]
29. Steilen-Gimbel H, Henn W, Kolles H, Moringlane JR, Feiden W, Steudel WI, et al. Early proliferation enhancement by monosomy 10 and intratumor heterogeneity in malignant human gliomas as revealed by smear preparations from biopsies. *Genes Chromosomes Cancer*. 1996; 16:180–184. [PubMed: 8814450]
30. Orndal C, Rydholm A, Willen H, Mitelman F, Mandahl N. Cytogenetic intratumor heterogeneity in soft tissue tumors. *Cancer Genet Cytogenet*. 1994; 78:127–137. [PubMed: 7828143]
31. Gorunova L, Hoglund M, Andren-Sandberg A, Dawiskiba S, Jin Y, Mitelman F, et al. Cytogenetic analysis of pancreatic carcinomas: intratumor heterogeneity and nonrandom pattern of chromosome aberrations. *Genes Chromosomes Cancer*. 1998; 23:81–99. [PubMed: 9739011]

32. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012; 366:883–892. [PubMed: 22397650]
33. Mor G, Yin G, Chefetz I, Yang Y, Alvero A. Ovarian cancer stem cells and inflammation. *Cancer Biol Ther*. 2011; 11:708–713. [PubMed: 21317559]
34. Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer Res*. 2005; 65:5506–5511. [PubMed: 15994920]
35. Wright MH, Calcagno AM, Salcido CD, Carlson MD, Ambudkar SV, Varticovski L. Brca1 breast tumors contain distinct CD44+/CD24– and CD133+ cells with cancer stem cell characteristics. *Breast Cancer Res*. 2008; 10:R10. [PubMed: 18241344]
36. Curley MD, Therrien VA, Cummings CL, Sergeant PA, Koulouris CR, Friel AM, et al. CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. *Stem Cells*. 2009; 27:2875–2883. [PubMed: 19816957]
37. Zhang S, Balch C, Chan MW, Lai HC, Matei D, Schilder JM, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. *Cancer Res*. 2008; 68:4311–4320. [PubMed: 18519691]
38. Silva IA, Bai S, McLean K, Yang K, Griffith K, Thomas D, et al. Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. *Cancer Res*. 2011; 71:3991–4001. [PubMed: 21498635]
39. Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, et al. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. *Cell Cycle*. 2009; 8:158–166. [PubMed: 19158483]
40. Rutella S, Bonanno G, Procoli A, Mariotti A, Corallo M, Prisco MG, et al. Cells with characteristics of cancer stem/progenitor cells express the CD133 antigen in human endometrial tumors. *Clin Cancer Res*. 2009; 15:4299–4311. [PubMed: 19509143]
41. Lou H, Dean M. Targeted therapy for cancer stem cells: the patched pathway and ABC transporters. *Oncogene*. 2007; 26:1357–1360. [PubMed: 17322922]
42. Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, et al. Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. *Proc Natl Acad Sci U S A*. 2006; 103:11154–11159. [PubMed: 16849428]
43. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006; 444:756–760. [PubMed: 17051156]
44. Ebben JD, Treisman DM, Zorniak M, Kutty RG, Clark PA, Kuo JS. The cancer stem cell paradigm: a new understanding of tumor development and treatment. *Expert Opin Ther Targets*. 2010; 14:621–632. [PubMed: 20426697]
45. Hermann DM, Kilic E, Spudich A, Kramer SD, Wunderli-Allenspach H, Bassetti CL. Role of drug efflux carriers in the healthy and diseased brain. *Ann Neurol*. 2006; 60:489–498. [PubMed: 17048260]
46. Bhola NE, Balko JM, Dugger TC, Kuba MG, Sanchez V, Sanders M, et al. TGF-beta inhibition enhances chemotherapy action against triple-negative breast cancer. *J Clin Invest*. 2013; 123:1348–1358. [PubMed: 23391723]
47. Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature*. 2006; 444:761–765. [PubMed: 17151667]
48. Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, Eberhart CG. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res*. 2006; 66:7445–7452. [PubMed: 16885340]
49. Tian F, Mysliwicz J, Ellwart J, Gamarra F, Huber RM, Bergner A. Effects of the Hedgehog pathway inhibitor GDC-0449 on lung cancer cell lines are mediated by side populations. *Clin Exp Med*. 2012; 12:25–30. [PubMed: 21519961]

50. Singh BN, Fu J, Srivastava RK, Shankar S. Hedgehog signaling antagonist GDC-0449 (Vismodegib) inhibits pancreatic cancer stem cell characteristics: molecular mechanisms. *PLoS One*. 2011; 6:e27306. [PubMed: 22087285]
51. Wang B, Liu J, Ma LN, Xiao HL, Wang YZ, Li Y, et al. Chimeric 5/35 adenovirus-mediated Dickkopf-1 overexpression suppressed tumorigenicity of CD44(+) gastric cancer cells via attenuating Wnt signaling. *J Gastroenterol*. 2013; 48:798–808. [PubMed: 23188090]
52. Chefetz I, Holmberg JC, Alvero AB, Visintin I, Mor G. Inhibition of Aurora-A kinase induces cell cycle arrest in epithelial ovarian cancer stem cells by affecting NFkB pathway. *Cell Cycle*. 2011; 10:2206–2214. [PubMed: 21623171]
53. Leizer AL, Alvero AB, Fu HH, Holmberg JC, Cheng YC, Silasi DA, et al. Regulation of Inflammation by the NF-kappaB Pathway in Ovarian Cancer Stem Cells. *American Journal of Reproductive Immunology*. 2011; 65:438–447. [PubMed: 20825380]
54. Alvero AB, Fu HH, Holmberg J, Visintin I, Mor L, Marquina CC, et al. Stem-like ovarian cancer cells can serve as tumor vascular progenitors. *Stem Cells*. 2009; 27:2405–2413. [PubMed: 19658191]
55. Green JM, Alvero AB, Kohen F, Mor G. 7-(O)-Carboxymethyl daidzein conjugated to N-t-Boc-hexylenediamine: a novel compound capable of inducing cell death in epithelial ovarian cancer stem cells. *Cancer Biol Ther*. 2009; 8:1747–1753. [PubMed: 19738422]
56. Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell*. 2009; 138:645–659. [PubMed: 19682730]
57. Naujokat C, Steinhart R. Salinomycin as a drug for targeting human cancer stem cells. *J Biomed Biotechnol*. 2012; 2012:950658. [PubMed: 23251084]
58. Yin G, Alvero AB, Craveiro V, Holmberg JC, Fu HH, Montagna MK, et al. Constitutive proteasomal degradation of TWIST-1 in epithelial-ovarian cancer stem cells impacts differentiation and metastatic potential. *Oncogene*. 2013; 32:39–49. [PubMed: 22349827]
59. Yin G, Chen R, Alvero AB, Fu HH, Holmberg J, Glackin C, et al. TWISTing stemness, inflammation and proliferation of epithelial ovarian cancer cells through MIR199A2/214. *Oncogene*. 2010; 29:3545–3553. [PubMed: 20400975]
60. Craveiro V, Yang-Hartwich Y, Holmberg JC, Sumi NJ, Pizzonia J, Griffin B, et al. Phenotypic modifications in ovarian cancer stem cells following Paclitaxel treatment. *Cancer Medicine*. 2013 n/a-n/a.
61. First World Report. View Points: Amgen looks to leapfrog rivals in Herceptin biosimilar race with Phase III trial in early stage breast cancer patients. *First World Report*; 2013.
62. Morris, JC.; Shapiro, GI.; Tan, AR.; Lawrence, DP.; Olencki, TE.; Dezube, BJ., et al. Phase I/II study of GC1008: A human anti-transforming growth factor-beta (TGFβ) monoclonal antibody (MAb) in patients with advanced malignant melanoma (MM) or renal cell carcinoma (RCC); ASCO Annual Meeting; 2008.
63. Bogdahn, U.; Hau, P.; Olyushin, V.; Mahapatra, AK.; Parfenov, V.; Venkataramana, NK., et al. Targeted therapy with AP 12009 in recurrent or refractory glioblastoma patients: Results of a phase IIb study; ASCO Annual Meeting; 2008.
64. Calvo-Aller E, Baselga J, Glatt S, Cleverly A, Lahn M, Arteaga CL, et al. First human dose escalation study in patients with metastatic malignancies to determine safety and pharmacokinetics of LY2157299, a small molecule inhibitor of the transforming growth factor-beta receptor I kinase. *ASCO Annual Meeting*. 2008
65. Gadir N, Jackson DN, Lee E, Foster DA. Defective TGF-beta signaling sensitizes human cancer cells to rapamycin. *Oncogene*. 2008; 27:1055–1062. [PubMed: 17700525]
66. Filyak Y, Filyak O, Stoika R. Transforming growth factor beta-1 enhances cytotoxic effect of doxorubicin in human lung adenocarcinoma cells of A549 line. *Cell Biol Int*. 2007; 31:851–855. [PubMed: 17418594]

Figure 1.

A proposed model of chemoresistance and targeted therapy against cancer stem cells