Entering an era of intelligent combination therapy in cancer

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The past few decades have witnessed unprecedented advances in our understanding of the molecular underpinnings of cancer. Although indiscriminately cytotoxic therapies like chemo- and radiation therapy remain standard of care for many cancer types, more precise targeted therapies and immune-boosting immunotherapies have added to our arsenal and afforded considerable survival gains.

Despite those advances, we are still no closer to a cure, particularly for the most aggressive and insidious cancers that progress rapidly or go undiagnosed until advanced stages of disease. The substantial genetic diversity of tumors and universal nature of drug resistance present the most formidable and enduring challenges to effective cancer treatment.

Researchers are proposing that the current treatment paradigm of administering single agents sequentially, with first-line therapy followed by second-line therapy with a different drug when the tumor inevitably relapses, is precluding any chance for a cure (Figure 1).[1] If the inherent challenges can be overcome, combination therapy may offer our best chance to stay one step ahead of cancer.

Picking the ideal partner

The Holy Grail for combination therapy is to identify drugs that have not just additive but *synergistic* antitumor efficacy – antitumor efficacy is magnified beyond what would be expected from the sum of the 2 drugs' effects – without significantly increasing the toxic side effects experienced by the patient.

The first step is to identify drug combinations that are most likely to work and to show compelling scientific and medical rationale for the combination. There are numerous ways of identifying potential combinations. Historically, hypothesis-driven approaches have been used; researchers identified drugs that should work well together because of their respective mechanism of action, based on our current understanding of the molecular underpinnings of cancer. Often there is also substantial preclinical evidence that the combination has synergistic antitumor activity in tumor cells.

As technology has become more sophisticated, more unbiased methods of identifying potential combinations have arisen. Chemical screening can reveal unexpected and promising combinations of existing drugs, while high-throughput genomic screening can identify interactions between potential drug targets, feedback loops within signaling pathways, and potential mechanisms of resistance that could all be targeted. More recently, computational modelling has emerged as a powerful tool for identifying potential drug combinations. Researchers are using Darwinian modeling and network and systems modeling to process large amounts of data in an effort to explain and predict therapeutic resistance and potential drug combinations.[2]

To assist in these preclinical efforts, major public repositories of protein pathway and network interaction pathways have been developed, including the Database of Interacting Proteins, maintained by researchers at the University of California Los Angeles, which documents experimentally determined protein interactions; and Pathway Commons, a network biology resource built and maintained by Memorial Sloan Kettering Cancer Center and the University of Toronto, as well as many others.[3,4]

The National Cancer Institute has created several resources to facilitate research on combination therapy. COMBO plates are 96-well plates containing all commercial anti-cancer drugs that have been approved by the US Food and Drug Administration (FDA) and which the NCI provides free for academic institutes, nonprofit organizations, and small businesses; NCI60 is a panel of 60 tumor cell lines that they provide to researchers to evaluate 2-drug combinations; and it has also created the Chemical Biology Consortium to facilitate drug discovery and development. The data derived from studies using these resources will be openly available to all researchers.[5,6]

Drug developers are also investing in improving our understanding of combination therapy. AstraZeneca recently partnered with the European

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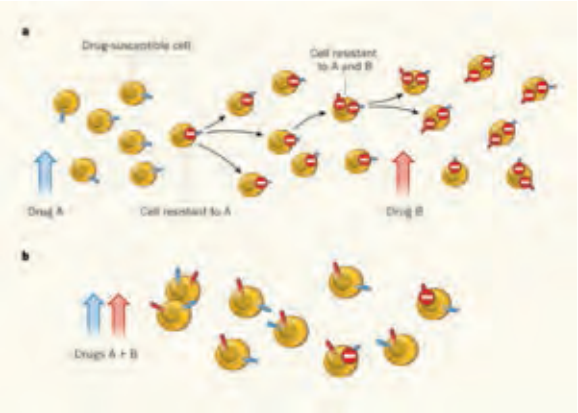

FIGURE 1 Two drugs are better than one: the mathematical rationale
According to a study, published in eLIFE by Ivana Bozic of Harvard University and colleagues, drug-resistant mutants typically exist at low levels in tumors prior to treatment. Monotherapy gives mutants that are resistant to that drug a competitive advantage and by the time second-line therapy commences, the chances that a mutant resistant to both drugs has already emerged are high. Using combination therapy from the beginning eliminates cells that are singly resistant to either drug and reduces the chances of a double mutant emerging.

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Bioinformatics Institute, the Sanger Institute, and Sage Bionetworks to launch a DREAM Challenge – these are "open innovation competitions and crowd-sourcing efforts designed to examine fundamental questions in biology and medicine." This particular challenge is seeking to understand the key traits of effective combination therapy and drug synergy using genomic data.[7]

Walking the walk

Swathes of recent exciting data on rationally designed combinations of immunotherapies and targeted therapies highlight the fact that the investment is paying off. Several combinations have now received regulatory approval in the United States (Table 1), and countless others are in various stages of clinical development. Currently, the most prominent combinations that have arisen from hypothesis-driven approaches have focused on several key scientific rationales, aimed predominantly at targeting the most common mechanisms of resistance to targeted therapies and immunotherapy.

Targeting one or more related RTK

Most successful molecularly targeted therapies are designed to inhibit receptor tyrosine kinases (RTKs), a family of more than 50 phosphorylating enzymes that orchestrate intracellular signaling pathways. A common mechanism of resistance to these drugs is the development of secondary mutations in the drug target that block its inhibition, or the compensatory activation of related RTKs. Therefore, targeting multiple RTKs or inhibiting the same receptor in more than one way can help to overcome resistance.

A prominent example of such a combination, and one of the first to be approved by the FDA, is the combination of pertuzumab and trastuzumab. Trastuzumab is a monoclonal antibody that targets the human epidermal growth factor receptor-2 (HER2), a protein that is highly overexpressed in breast cancer, that has become standard of care for patients with HER2-overexpressing disease in combination with chemotherapy. Pertuzumab also targets the HER2 protein, but binds to a different domain on the receptor than trastuzumab, blocking ligand-dependent HER2 dimerization with other members of the HER family, whereas trastuzumab inhibits ligand-independent HER2 signaling. The combination is hypothesized to produce a more complete inhibition of HER2 signaling pathways.[8-10]

In 2012 the FDA granted accelerated approval to pertuzumab in combination with trastuzumab and docetaxel as first-line therapy in patients with HER2-positive metastatic breast cancer, followed in 2013 in patients with HER2-positive, locally advanced, or early stage breast cancer. The latter was based on the demonstration of improved pathologic complete response (pCR) rates in a randomized, phase 2 trial[11] and a phase 3 trial seeking to establish a survival benefit in this setting is ongoing (NCT02586025).

In the metastatic setting, approval was based on the demonstration of improved progression-free survival (PFS) in the phase 3 CLEOPATRA trial.[12] Follow-up data for this study at a median of 50 months were recently published and demonstrated improved median overall survival (OS) of 15.7 months (hazard ratio [HR], 0.68; $P < .001$) and a 7.7-month improvement in duration of response (HR, 0.68), with no effect on long-term cardiac safety.[13] An analogous phase 3 trial is ongoing in gastric cancer (the JACOB trial; NCT01774786).

Vertical targeting within a signaling pathway

Another common mechanism of resistance to targeted therapies is the activation of downstream components of the targeted signaling pathway, to bypass inhibition of the upstream kinase. Since drugs targeting a variety of points in numerous different pathways are now in clinical development, combining drugs targeting RTKs with those targeting downstream effectors is a viable strategy. Two such combinations have recently received regulatory approval.

Activating mutations in the BRAF kinase are present in about 40%-60% of advanced melanomas, most commonly a *V600E/K* mutation.[14,15] Potent inhibitors of BRAF have

TABLE 1 FDA-approved molecularly targeted drug combinations

Drugs	Mechanism of action	Manufacturers	Approved indication
Ipilimumab (Yervoy) + nivolumab (Opdivo)	Immune checkpoint inhibitors; monoclonal antibodies targeting CTLA-4 and PD-1	Bristol-Myers Squibb	Unresectable/metastatic melanoma
Palbociclib (Ibrance) + letrozole (Femara)	CDK4 inhibitor + aromatase inhibitor	Pfizer Novartis	ER-positive, HER2-negative advanced breast cancer
Lapatinib (Tykerb) + letrozole (Femara)	Dual inhibitor of EGFR and HER2 + aromatase inhibitor	GlaxoSmithKline Novartis	Postmenopausal women with HR-positive metastatic breast cancer
Pertuzumab (Perjeta) + trastuzumab (Herceptin)	Monoclonal antibodies targeting HER2	Genentech	HER2-positive, locally advanced or early stage breast cancer; in combination with docetaxel chemotherapy
Trametinib (Mekinist) + dabrafenib (Tafinlar)	MEK inhibitor + BRAF inhibitor	GlaxoSmithKline	*BRAF V600E/K* mutation-positive, unresectable or metastatic melanoma
Carfilzomib (Krypolis) + lenalidomide (Revlimid)	Proteasome inhibitor + immunomodulatory agent	Onyx Celgene	Relapsed multiple myeloma

CDK4, cyclin-dependent kinase 4; CTLA-4, cytotoxic T-lymphocyte antigen-4; FDA, Food and Drug Administration; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; MEK, mitogen-activated protein kinase kinase; PD-1, programmed cell death 1

been developed and are approved by the FDA for the treatment of patients with *BRAF V600E/K*-mutant metastatic melanoma, demonstrating dramatic responses as single agents,[16,17] though these responses are short lived as resistance inevitably develops. Most reported resistance mechanisms involve reactivation of the mitogen-activated protein kinase (MAPK) pathway, downstream of BRAF, thus targeting a component of that downstream signaling – mitogen activated protein kinase kinase (MEK) – could help to overcome resistance.[18]

That hypothesis bore out when the combination of the BRAF inhibitor dabrafenib and MEK inhibitor trametinib proved more effective than dabrafenib alone. The combination was granted accelerated approval by the FDA in early 2014 based on the demonstration of durable objective responses in a randomized phase 2 trial.[19] The FDA is currently reviewing an application for full approval based on data from the phase 3 COMBI-d and COMBI-v studies, which showed improved OS, with a decision expected later this month.[20,21]

Another example of an FDA-approved vertical targeting strategy involves the addition of a cyclin-dependent kinase (CDK) inhibitor to an aromatase inhibitor (AI) in breast cancer. Palbociclib is an inhibitor of CDK4/6, serine/threonine kinases that play a key role in regulating the transition between phases of the cell cycle, which are frequently dysregulated in cancer to allow the cancer cell to continuously enter the cell cycle, driving uncontrolled proliferation.

CDKs are a downstream target of estrogen receptor (ER) activation and may represent a mechanism of resistance to endocrine therapies, like the AI letrozole, which blocks the conversion of androgen into estrogen and reduces circulating levels of the latter to block the cancer growth-promoting effects of this hormone in ER-overexpressing tumors. CDK inhibitors are undergoing clinical development, most prominently in breast cancer, and preclinical trials showed synergistic activity with letrozole.[22]

The combination of palbociclib and letrozole was approved in February 2015 for first-line treatment of postmenopausal women with ER-positive, HER2-negative advanced breast cancer, based on the demonstration of improved PFS compared with letrozole alone in the phase 2 PALOMA-1 trial.[23]

Horizontal targeting across parallel pathways

Focusing on single pathways is somewhat of an oversimplification and, in reality, cancer biologists have uncovered complex signaling networks, with significant cross-talk, interaction, and feedback loops between signaling pathways. Interruption of one signaling pathway using a targeted agent can lead to compensatory activation of parallel pathways that can drive resistance.[24] As a result, combining agents that target these different pathways could prove promising.

Indeed, 2 agents that target key pathways in the development of breast cancer – the HER2 and ER pathways – have received regulatory approval. The dual-targeting HER2/epidermal growth factor receptor (EGFR) inhibitor lapatinib was approved in combination with letrozole in 2010 for the treatment of postmenopausal women with HER2 and HR-positive advanced breast cancer.[25]

Combination immunotherapy

Immunotherapy has emerged as one of the most promis-

ing anticancer strategies in recent years, particularly in the case of immune checkpoint inhibitors, a type of immunotherapy that activates cancer-fighting T-cells by inhibiting key proteins on the surface of these cells that function like molecular brakes, and thus effectively kick-starting the immune system. Impressive, durable responses have been observed in a variety of tumor types and, since 2011, the list of FDA-approved immune checkpoint inhibitors has been growing steadily.[26] Incorporating these agents into combination therapy, either with other immunotherapies or with targeted agents, could yield even more impressive results, with curative potential.

Indeed, in October, the first combination immunotherapy was approved by the FDA for the treatment of *BRAF*-wildtype metastatic melanoma; nivolumab and ipilimumab are both immune checkpoint inhibitors each with a nonredundant mechanism of action on the immune system – nivolumab targets programmed cell death 1 (PD-1) and ipilimumab targets cytotoxic T-lymphocyte antigen 4 (CTLA-4) – that are already approved as single agents in this disease setting and in metastatic squamous non-small-cell lung cancer in the case of nivolumab.[27]

Accelerated approval of this combination was based on the results of the phase 2 CheckMate-069 trial, which were presented at the 2015 annual meeting of the American Association for Cancer Research (AACR).[28] The response rate was 60%, including 17% complete response (CR) and 43% partial response (PR), compared with 11% in patients treated with ipilimumab alone. Promising efficacy has subsequently been observed in the phase 3 CheckMate-067 trial; among 945 patients the median PFS was 11.5 months in the combination arm, compared with 2.9 months and 6.9 months for ipilimumab and nivolumab alone, respectively. There was a higher rate of treatment-related AEs for the combination therapy, but no unexpected toxicities occurred.[29] This data have now been submitted to the FDA to support an application for full approval, with a decision expected in early 2016.

The combination of ipilimumab and nivolumab is being examined in a number of phase 3 trials in other cancer types, as is the combination of tremelimumab (a CTLA-4 inhibitor) and MEDI4736 (a programmed cell death ligand 1 [PD-L1] inhibitor) (Table 2). Other immune checkpoint inhibitors are also undergoing earlier phases of clinical development in a plethora of potential combinations, including with other immune checkpoint inhibitors, with other types of immunotherapy, and with targeted therapies. The results of a phase 1 trial of tremelimumab in combination with the immunostimulatory anti-CD40 antibody CP-870,893 were reported at AACR this year and found the combination to be safe and, over a median follow-up of 22 months, the ORR was 27%, including 2 CR and 4 PR.[30]

Addressing the challenges of combination therapy

Combination therapy is not without its challenges, most significantly the potential for additive toxicity, and identifying patients who are most likely to respond to a particular combination will be key. As yet, the lack of biomarkers, assays and imaging tools is a major hurdle to their effective development. The cost of combination therapy is another important issue – both in terms of the investment required to develop them and the cost to the patient of the final approved therapies. The latter is drawing substantial scrutiny, with the yearly average cost of ipilimumab and nivolumab combination therapy estimated at $256,000.[31] There are also regulatory challenges to developing combination therapies, although regulatory agencies are now trying to address these by accommodating greater flexibility and issuing guidance.[2,6]

TABLE 2 Phase 3 clinical trials of immune checkpoint inhibitor combination therapy

Combination	Mechanism of action	Manufacturers	Indication (clinicaltrials.gov identifier)
Tremelimumab + MEDI4736	Monoclonal antibodies targeting CTLA-4 and PD-L1	Pfizer MedImmune	Head and neck cancer (KESTREL - NCT02551159; NCT02369874) Bladder cancer (NCT02516241) NSCLC (MYSTIC – NCT02453282; NEPTUNE – NCT02542293; ARCTIC – NCT02352948)
Ipilimumab (Yervoy) + nivolumab (Opdivo)	Monoclonal antibodies targeting CTLA-4 and PD-1	Bristol-Myers Squibb	Renal cell carcinoma (CheckMate214; NCT02231749) Glioblastoma (CheckMate143; NCT02017717) Small-cell lung cancer (CheckMate451; NCT02538666) NSCLC (CheckMate227; NCT02477826) Melanoma (NCT02339571)
Pembrolizumab (Keytruda) + lenalidomide (Revlimid)	Monoclonal antibody targeting PD-1 + immunomodulatory agent	Merck Celgene	Multiple myeloma (KEYNOTE-185; NCT02579863)

CTLA-4, cytotoxic T-lymphocyte antigen-4; NSCLC, non-small cell lung cancer; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1

Since the constituent drugs of combination therapy are often being developed by different pharmaceutical companies, developing combination regimens requires cooperation, which can often be limited by commercial factors, including intellectual property claims. However, companies are becoming increasingly open to working with their competitors and developing ways to move past these obstacles. A prominent example is Pharmacyclics, which developed the Bruton's tyrosine kinase inhibitor ibrutinib in collaboration with Janssen Biotech and which recently announced that it had partnered in clinical trials with the makers of other promising anticancer drugs, including AstraZeneca, Bristol-Myers Squibb, Celgene, and Roche.

Ibrutinib is already approved to treat chronic lymphocytic leukemia (CLL) as a single agent, but is also showing significant promise in combination therapy. Furthest along in development are strategies pairing ibrutinib with the anti-CD20 antibody rituximab (R), alone and in combination with bendamustine (B) chemotherapy. The results of the phase 3 HELIOS trial, evaluating the combination of ibrutinib and BR in patients with previously treated CLL or small lymphocytic leukemia, were recently presented at the annual meeting of the American Society of Clinical Oncology. At a median follow-up of 17.2 months, median PFS was not yet reached in the combination arm, compared with 13.3 months in the BR arm. AEs were similar in the two arms, with higher rates of grade 3/4 atrial fibrillation and major hemorrhage in the combination arm.[32]

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