Improving combination cancer therapy: the CombiPlex[®] development platform

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Current combination therapy approaches assume that better outcomes are achieved by combining drugs at their maximally tolerated doses. However, administration of individual agents cannot consistently deliver synergistic drug ratios to tumor cells due to differences in pharmacokinetics of the individual drugs. Further, the toxicity of combination regimens often necessitates administration of suboptimal dosages. Delivery technologies, such as the CombiPlex[®] platform, can enable efficient and sustained delivery of combination treatments at a synergistic ratio. The CombiPlex platform determines synergistic drug ratios *in vitro* and identifies an appropriate nanoscale carrier to maintain that ratio *in vivo* and enhance its delivery to tumor cells. CPX-351, a dual-drug liposomal encapsulation of cytarabine and daunorubicin, is the first clinical proof-of-concept example of the CombiPlex platform.

First draft submitted: 7 November 2017; Accepted for publication: 8 January 2018; Published online: 24 January 2018

Keywords: combination chemotherapy • CPX-351 • liposomes

Combination therapy is essential for many effective treatments for cancer, given the multiple pathways by which cancers grow and resist therapies [1,2]. However, toxicities often necessitate the administration of suboptimal doses of many of the agents given as part of combination regimens [3], and antagonistic activities and discordant pharmacokinetics of co-administered agents can potentially lead to reduced therapeutic activity.

This review describes challenges inherent in the current process for developing combination cancer therapy. It emphasizes the critical role that synergistic molar ratios of drug combinations may play in enhancing the efficacy of

combination treatments. It also discusses ways in which early (e.g., preclinical) development that incorporates the use of carefully selected delivery technologies can improve the effectiveness of combination therapy development, resulting in improved patient outcomes. CombiPlex[®], a combination drug technology platform, and its proof-of-concept drug, CPX-351, are presented herein as an example of the way in which technology can be used to develop better combination therapies.

Challenges associated with current combination cancer therapy development

In the 1960s, clinical studies of pediatric leukemia patients demonstrated that the efficacy of single-agent therapies could be dramatically enhanced by combining drugs with different mechanisms of action during the course of treatment [1]. In recent years, a number of targeted antitumor agents (i.e., bevacizumab, cetuximab and erlotinib) have shown limited anticancer activity as single agents but have enhanced efficacy when combined with conventional chemotherapy [1]. Compared with single-agent therapies, determining the optimal dosing and dosing schedule of each agent is of greater consequence for combination regimens.

Currently, typical combination regimens consist of two or more antineoplastic agents chosen to simultaneously disrupt multiple mechanisms associated with tumor growth [1]. Although the advent of combination therapy has improved outcomes for individuals with some cancers, many challenges remain [4]. For some cancers, the use of existing multidrug regimens seldom leads to durable tumor remission [4]. Combination chemotherapy also remains limited by toxicity to normal healthy cells and tissues, as well as by the inability to coordinate drug pharmacokinetics and tumor exposure in order to minimize the development of multidrug resistance [4]. Furthermore, despite the increased target specificity and potency of new molecularly targeted agents, many combinations of such drugs also require simultaneous target inhibition to be effective and, consequently, share challenges related to coordinated pharmacokinetics and toxicities to healthy tissues [4].

The traditional approach to drug combination development and assessment fails to recognize the critical role that molar ratios of combined drugs play in enhancing the efficacy of therapeutic agents [1,5]. The optimally efficacious exposure of many combinations may not be equivalent to the maximum-tolerated dose (MTD) of the individual agents, and the doses recommended for combination therapy are often empirically derived in clinical trials [5]. The efficacy of combinations requires temporal control of drug exposure to ensure that the degree of target inhibition and/or interaction is coordinated in appropriate tissues [5]. Taking advantage of these relationships requires that drug ratios be controlled following administration [5]; however, it is extremely difficult, if not impossible, to coordinate the pharmacokinetics of concurrently administered, conventional, free (nonencapsulated) drugs due to differences in the agents' clearance, tissue uptake and metabolism.

Development of individual drugs leading to combination regimens

In nearly all instances, the development of new drug combinations continues to be based on increasing the dose of each drug to the MTD, using the monotherapy dosing schedule of each individual drug (Figure 1A) [1,4,6]. *In vitro* and *in vivo* preclinical studies may be conducted to support the utility of two or more drugs in combination, but each drug is typically developed individually until registration [5]. Pharmacokinetic and pharmacodynamic properties are optimized to produce the most efficacious single treatment regimen possible. Early in the development process, an individual drug's pharmacokinetic and pharmacodynamic profiles are rarely studied together with those of the agent(s) with which that drug is likely to be combined. By the time combination clinical studies are initiated, the constituent agents may have very different treatment schedules, necessitating extensive adjustment during initial clinical trials of the combination [5]. In theory, the constituents of combination therapies should be developed together beginning at the preclinical stage, without relying heavily on the agents' monotherapy dosages and schedules (Figure 1B) [5]. However, it is important to note that while this approach to drug development is theoretically sound, it has not yet been validated and not all efforts using this strategy have been successful.

Drug ratio dependency

Many drug combinations require simultaneous exposure for effective antitumor activity, suggesting interrelated mechanisms of action [1]. The implication of this is that the relative concentration of each agent exposed to the cancer cell (molar drug ratio) is important. Drug combinations may act synergistically, additively or antagonistically, depending on the molar ratios of the individual agents comprising the combination [7,8]. Additive drug ratios result in activity that is equal to the sum of the effects contributed by each drug [6]. Synergistic ratios result in activity that is greater than simply the added effect of the individual drugs [1], while antagonistic ratios result in activity that is

Figure 1. Possible pathways for preclinical and clinical development of anticancer drug combination treatment regimens. (A) The traditional pathway, in which the constituents of the combination are tested separately through Phase II and then re-tested in combination Phase I and Phase II studies. **(B)** An example of the rational development of a drug combination product using the CombiPlex® platform, with which only the combination product, not its individual constituents, is assessed in Phase I and Phase II studies. MTD: Maximum-tolerated dose. Data taken from [5].

less than the expected additive effect of the individual drugs, although some level of activity typically remains [9]. According to the ratiometric dosing hypothesis, delivery of synergistic molar drug ratios to target tissues/cells may enhance treatment efficacy [8,9]. For example, *in vitro* experiments identified a strong antagonistic effect between irinotecan and cisplatin when they were administered at a 1:1 ratio, but a synergistic effect was observed at a ratio of 4:1. In contrast, the combination of irinotecan and floxuridine was synergistic at an equimolar ratio but was strongly antagonistic at a 10:1 ratio. Once a synergistic ratio is identified *in vitro*, maintaining that ratio *in vivo* is crucial. For instance, a ratio of 4:1 was identified as synergistic *in vitro* for a dual-drug liposomal formulation of doxorubicin and vincristine. However, once encapsulated, vincristine leaked from the liposome considerably, leading to an antagonistic 20:1 drug ratio that was less effective than free drug [10].

Variable drug pharmacokinetics

With conventional combination regimens, even if one simultaneously administers the free (nonencapsulated) drugs, it is virtually impossible to coordinate ideal exposure of the combined agents in target tissues and/or cancer cells due to differences in the elimination, metabolism and pharmacokinetic properties of the individual drugs (Figure 2A) [5]. Dissimilar pharmacokinetic profiles result in constantly changing drug ratios after dosing, thus preventing the maintenance of any particular drug ratio that could potentially synergize antitumor activity [1,9]. To compensate for these pharmacokinetic differences, combination regimens often continuously expose the patient to high doses of both agents in an attempt to achieve simultaneous and prolonged inhibition of each target [5]. This strategy can lead to extensive exposure of healthy tissues to the drugs, resulting in unnecessary toxicity that can compromise the utility of the combination regimen [5].

Drug exposure can also vary between tissues due to local differences in drug metabolism and elimination [5]. Failure to control drug ratios *in vivo* due to uncoordinated pharmacokinetics has the potential to render tumors

Figure 2. Drug concentrations following intravenous administration of either a combination of free cytarabine and daunorubicin (A) or CPX-351 dual-drug liposome of cytarabine plus daunorubicin (B) to rats. Quantitative whole body autoradiography images of radiolabeled (^{14}C) cytarabine (top) and daunorubicin (bottom); images are shown for 1 h (left) and 24 h (right) after intravenous administration to Sprague–Dawley rats. Darker shades represent increased drug concentration.

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more prone to drug resistance, since compensatory survival signaling within tumor cells may be triggered when exposure to one of the agents falls below a therapeutically active concentration threshold [9].

Clinical examples of drug combinations

Our increasing understanding of the molecular lesions that initiate the formation of cancerous cells and cause them to be resistant to treatments, along with the advent of highly potent and specific agents targeted against these molecular lesions, has generated high expectations that combination treatment regimens would be ‘revolutionized’, leading to numerous breakthroughs in cancer patient outcomes. While there have been notable successes in this regard, there are many examples of molecularly targeted drug combinations that were theoretically attractive based on complementary mechanisms of action, but that proved to be unsuccessful due to limited efficacy, tolerability issues or both (Table 1). For example, the closely related VEGF and EGFR pathways share common signaling components and are both involved in tumor growth and progression [11]. Both VEGF and EGFR inhibitors have demonstrated antitumor activity as single agents; however, co-inhibition of the two pathways (with bevacizumab and erlotinib, respectively) in combination with chemotherapy has not been shown to improve efficacy in patients with non-small-cell lung cancer, despite promising results from Phase I and II studies [11,12]. In patients with

Table 1. Overview of combination therapy studies.

Author (year)	Targeted pathways	Combination	Efficacy (combination vs control, respectively)	Safety (combination vs control, respectively)	Malignancy	Ref.
Herbst <i>et al.</i> (2011)	VEGF and EGFR	Bevacizumab + erlotinib	OS: 9.3 vs 9.2 months PFS: 3.4 vs 1.7 months ORR: 6 vs 13%	Serious AE rate: 42 vs 36%	NSCLC	[12]
Tol <i>et al.</i> (2009)	VEGF and EGFR	Cetuximab + bevacizumab + chemotherapy	OS: 19.4 vs 20.3 months PFS: 9.4 vs 10.7 months ($p = 0.01$) ORR: 53.7 vs 50.0%	Grade 3/4 AE rate: 81.7 vs 73.2% ($p = 0.006$)	CRC	[13]
Hecht <i>et al.</i> (2009)	VEGF and EGFR	Panitumumab + bevacizumab + chemotherapy	OS: 19.4 vs 24.5 months PFS: 10.0 vs 11.4 months	Grade 3/4 AE rate: higher in the panitumumab arm than control	CRC	[14]
Harshman <i>et al.</i> (2013)	VEGF and mTOR	Bevacizumab + everolimus	PFS: 5.1 vs 6.0 months (historic control) OS: 21.0 months	Four of ten pts discontinued due to toxicity	RCC	[15]
Alvarez <i>et al.</i> (2013)	VEGF and mTOR	Bevacizumab + temsirolimus	OS: 16.9 months PFS: 5.6 months ORR: 24.5%	38.8% of pts discontinued due to toxicity	Endometrial cancer	[16]
Enggebraaten <i>et al.</i> (2012)	EGFR and MT polymerization	Gefitinib + docetaxel	ORR: One vs four PR	Study was closed after discontinuation of seven (three in gefitinib arm and four in control arm) of 16 pts due to toxicity	Breast cancer	[17]
Tolcher <i>et al.</i> (2015)	MEK and Akt-1, 2 and 3	Selumetinib + MK-2206	ORR: 23% of NSCLC pts and one of two ovarian cancer pts	Substantial dose and schedule modifications required due to AEs	Solid tumors	[18]
Shoushtari <i>et al.</i> (2016)	Akt and MEK	Trametinib + GSK2141795	PFS: 15.6 vs 15.7 weeks	Seven of 18 pts required dose reductions due to AEs	Uveal melanoma	[19]

AE: Adverse event; CRC: Colorectal cancer; MT: Microtubule; NSCLC: Non-small-cell lung cancer; ORR: Overall response rate; OS: Overall survival; PFS: Progression-free survival; PR: Partial response; pt: Patient; RCC: Renal cell carcinoma.

metastatic colorectal cancer, the combination of anti-VEGF and anti-EGFR agents (bevacizumab and cetuximab or panitumumab, respectively) with chemotherapy was associated with increased toxicity and decreased progression-free survival, further suggesting that co-inhibition of the VEGF and EGFR pathways in addition to chemotherapy may not be an effective strategy [13,14].

In Phase II studies, combining the VEGF-A inhibitor bevacizumab with a mTOR inhibitor (everolimus in patients with refractory metastatic renal cell carcinoma [15] and temsirolimus in women with recurrent or persistent endometrial cancer [16]) resulted in unacceptable toxicity. The combination of the EGFR inhibitor gefitinib with the chemotherapy drug docetaxel in a Phase II study in patients with metastatic breast cancer resulted in unexpected toxicity; the combination treatment was associated with dehydration and electrolyte disturbances not seen when the agents were administered as monotherapy [17]. A Phase I study that enrolled patients with locally advanced or metastatic solid tumors (MTD finding period) and *KRAS*-mutant non-small-cell lung cancer (MTD expansion period) illustrates the difficulties that often arise when a combination of agents is not tolerable [18]. In this trial, a non-ATP-competitive MEK inhibitor (selumetinib) was combined with an Akt-1, -2 and -3 inhibitor (MK-2206) [18]. Previous studies that separately characterized the pharmacokinetic-pharmacodynamic profile of each drug had determined that MTDs were 60 mg once every other day or 200 mg weekly for MK-2206 and 75 mg twice daily for selumetinib [18]. With separate dosing, clinical toxicities were mainly erythematous maculopapular rash and stomatitis for MK-2206 and acneiform dermatitis, diarrhea, fatigue, nausea and peripheral edema for selumetinib. Rash was a dose-limiting toxicity for both drugs [18]. Following dose and schedule adjustments, recommended study combination drug doses were 135 mg once weekly for MK-2206 and 100 mg once daily for selumetinib [18]. A separate Phase II study similarly found that the addition of the Akt inhibitor GSK2141795 to the MEK inhibitor trametinib necessitated many dose reductions for adverse events in patients with advanced uveal melanoma, without improvement in progression-free survival [19]. The poor tolerability of these combination regimens may be due to overlapping toxicities and/or unexpected interaction between the drugs' mechanisms of action.

Despite high expectations, these combinations showed inconsistent activity, which might be due to the multiple challenges of combining these molecularly targeted agents [18]. There are no standardized trial designs for assessing combination regimens, and permutations of dose and schedule result in many complexities. In some instances, such as with the combination of MEK and Akt inhibitors, overlapping toxicities necessitate de-escalation of the doses of both agents to improve tolerability. Further, it is often not possible to determine whether the heterogeneity of response is attributable to the complexities of tumor biology, presence of driver mutations, disruption of signaling feedback loops, poor tumor drug penetration, suboptimal/uncoordinated exposure to the combined agents (e.g., drug ratio) or several of these factors. Furthermore, trials of new combinations are often small and not powered for the evaluation of patient characteristics that may influence response and toxicity.

Using nanoparticles to deliver drug combinations may address some of these factors, specifically suboptimal exposure and poor target penetration. Drugs within a nanoparticle take on the pharmacokinetic characteristics of their carrier, allowing for greater coordination of drug exposure. In addition, the uptake of a nanoparticle can strongly influence the biodistribution of the encapsulated drugs, providing greater access to target tissues. Several single-agent chemotherapy drugs may currently be delivered via liposome and solid-core nanoparticles, including doxorubicin (Doxil®), daunorubicin (DaunoXome®), paclitaxel (Abraxane®), tamoxifen, methotrexate and camptothecin. Nanoparticles can also deliver hydrophobic, molecularly targeted agents, such as the Akt inhibitor ipatasertib [20]. While combination therapy can be achieved using discrete nanoparticles for each drug, coformulation of both drugs within the same nanoparticle allows for coordinated pharmacokinetic behavior that can facilitate targeted delivery of a synergistic drug:drug ratio.

Improving combination therapy development processes

The goals of rational, prospectively developed combination therapies are to improve efficacy by delivering a synergistic drug ratio to tumor tissues and/or cancer cells for prolonged periods of time, while avoiding harmful effects by limiting systemic exposure to healthy tissues [6,8]. The CombiPlex platform is an example of such a technology-based approach to the development of drug combinations that involves a system of dual-drug screening, nanoscale formulation and preclinical evaluation to make the development process more efficient, while also resulting in improved patient outcomes [5]. With CombiPlex, drug ratio and tumor exposure are evaluated for drugs in various fixed ratio combinations at the preclinical stage, making it possible to begin clinical trials with a combination treatment that has been prospectively evaluated for therapeutic synergy. During the drug screening

phase, drug ratios are identified that exhibit synergistic activity, with minimal antagonistic activity, against a range of cancer cell lines.

Once a synergistic ratio has been identified with the CombiPlex platform, nanoscale drug carriers, such as liposomes and polymeric nanoparticles, are utilized to coordinate the pharmacokinetics and tissue exposure profiles of both agents (Figure 2B), maintain the drug ratio over an extended period of time and preferentially deliver drugs at the desired ratio to tumor cells [1,6]. Nanoscale drug carriers are particularly well suited to controlling drug ratios (via encapsulation) during the drug delivery process [1,6], a feature that cannot be achieved via co-administration of free agents due to the drugs' uncoordinated distribution, metabolism and elimination [5]. Thus, advanced drug carriers may enable synergistic drug combinations in the preclinical setting, in a manner that can be translated more predictably into the clinic [5]. In most instances, the nanoscale drug carrier can be targeted for preferential uptake into tumor tissues or cancer cells, while also limiting exposure in normal healthy tissues [6]. These attributes of the CombiPlex platform help to overcome many of the problems associated with conventional combination regimens, in which the therapeutic window between efficacious and toxic doses is often very narrow [5]. They also ensure that cancer cells are exposed to synergistic drug ratios at sufficient concentrations to be highly efficacious [5].

Since the CombiPlex process establishes synergistic drug ratios and tumor exposure during the preclinical stage, clinical studies can be initiated with the drug combination product at the first-in-man stage [5]. This is possible because the pharmacokinetic and distribution properties of appropriately designed small (<100 nm diameter) liposomes and nanoparticles typically scale allometrically across mammalian nonclinical models to man. Consequently, it may be possible to arrive at a definitive 'Go/No-Go' decision with a CombiPlex-derived combination product more quickly than with a product developed using the traditional combination development model [5].

As described in the following section, results from multiple clinical trials have demonstrated that the CombiPlex platform is able to translate the identification of a synergistic drug ratio in a preclinical setting to the development of combination regimens with improved clinical efficacy [4,21–23].

CPX-351: a CombiPlex-derived therapy for acute myeloid leukemia

CPX-351 is a CombiPlex-derived dual-drug liposomal combination of daunorubicin and cytarabine at a synergistic drug ratio [24], and was approved in August 2017 by the US FDA for the treatment of adults with newly diagnosed therapy-related acute myeloid leukemia (AML) or AML with myelodysplasia-related changes [25]. The liposome composition maintains a synergistic ratio for over 24 h after intravenous injection in the plasma [26]. In animal models, CPX-351 has been shown to accumulate and persist for days in bone marrow. Within the bone marrow compartment, CPX-351 has also been shown to be taken up to a greater extent by leukemia cells than normal bone marrow cells [24,27]. Together, the effects of prolonged bone marrow exposure to the synergistic drug ratio and preferential uptake of CPX-351 by leukemia cells lead to marked improvements in effective antileukemia activity (Figure 3).

CPX-351 rationale

For over 40 years, combination chemotherapy incorporating cytarabine plus an anthracycline, such as daunorubicin (7 + 3 regimen), has remained the standard of care for AML [28]. In that time, the dose and schedule of the 7 + 3 regimen was refined to reduce toxicity while maintaining efficacy [29]. Attempts to improve the clinical activity of the cytarabine/daunorubicin combination by administering high-dose cytarabine [30,31] or modifying the anthracycline dose and schedule [32] did not improve survival and in some instances increased the toxicity of the combination.

While optimization of the dose and dosing schedule of the 7 + 3 regimen took decades, the CombiPlex approach allows for a more efficient development process, as refinement of the pharmacokinetic profile can occur *in vitro*, reducing the time spent on clinical development. In contrast to the 7 + 3 regimen, CPX-351 was designed by rationally rethinking the drug combination as a single entity, leading to its notable activity and comparatively quick FDA approval. An approximate 5:1 molar ratio of cytarabine and daunorubicin was found to have maximally synergistic and minimally antagonistic antileukemia activity *in vitro* compared with other tested ratios (Figure 4A) [24]. An excess of intracellular cytarabine is important due to the drugs' individual mechanisms of action. To preserve the molar drug ratio and prevent independent and dissimilar elimination and metabolism of cytarabine and daunorubicin, CPX-351 encapsulates the two drugs within a 100 nm bilamellar liposome [27]. Liposomal encapsulation markedly increases the plasma half-life of cytarabine and daunorubicin [26,33] and results in increased exposure within the bone marrow at a synergistic drug ratio [34–36]. *In vivo* studies of drug ratio dependency

Figure 3. Schematic representation of CPX-351 (cytarabine:daunorubicin) liposome injection. The liposomes are bilamellar, and the outer vesicle has a diameter of 100 nm. The membrane is composed of DSPC:DSPG:cholesterol in a 7:2:1 molar ratio. The active agents, cytarabine and daunorubicin, are encapsulated in the aqueous space of both vesicles at a 5:1 molar ratio. Liposomes are suspended in unbuffered sucrose. While inside the liposome, daunorubicin is complexed with copper as copper gluconate, giving CPX-351 a deep purple color. Copper gluconate stabilizes retention of daunorubicin inside the liposome and allows the 5:1 drug ratio to be maintained until delivery to the bone marrow/leukemic cells. The strength of CPX-351 is 5 units/ml, where 1 unit equals 1.0 mg cytarabine plus 0.44 mg daunorubicin (base).
 DSPC: Distearylphosphatidylcholine; DSPG: Distearylphosphatidylglycerol.

using the P388 murine leukemia model showed that the day 55 survival rate of BDF-1 mice was highest with the 5:1 cytarabine:daunorubicin ratio (Figure 4B). In fact, this drug ratio provided increased efficacy over other ratios in which substantially more drug was administered [24]. Since CPX-351 is a product of the CombiPlex platform, the cytarabine:daunorubicin drug ratio and tumor exposure were designed for synergistic activity at the preclinical stage, making it possible to begin CPX-351 clinical trials with the synergistic combination at the first-in-man stage.

Clinical experience with CPX-351 in acute myeloid leukemia

The CPX-351 clinical program in AML is summarized in Table 2. The Phase I study identified a recommended dose of 101 units/m² (delivering 101 mg/m² cytarabine and 44 mg/m² daunorubicin) for later studies and determined that the 5:1 cytarabine:daunorubicin molar ratio is maintained in plasma for up to 24 h, and observed strong signs of clinical activity in the form of complete remissions in patients with advanced previously treated hematologic malignancies [26].

A Phase II clinical trial (NCT00788892) [37] evaluated CPX-351 versus standard 7 + 3 chemotherapy in older patients (60–75 years of age) with newly diagnosed AML. Overall, CPX-351 was associated with a higher response rate than the 7 + 3 regimen (66.7 vs 51.2%; $p = 0.07$, meeting the predetermined criterion for success [$p < 0.1$]) and a lower 30-day induction mortality [37]. A trend toward prolonged overall and event-free survival was observed in the overall study population. Significant differences favoring CPX-351 were also observed for the response rate in patients with high-risk cytogenetics and secondary AML (sAML), as well as for 24-month event-free survival and overall survival in patients with sAML (Table 2 & Figure 5) [37]. These findings are notable because AML patients with high-risk subtypes, including sAML [41,42] and older age [43], typically have a poor prognosis and are particularly difficult to treat. It is important to note that this study evaluated the benefit of CPX-351 relative to conventional chemotherapy, and not the absolute activity of CPX-351. It is likely that a larger patient population would be needed to distinguish any benefit of CPX-351 to patients with *de novo* disease, since those patients have a more robust response to conventional chemotherapy.

Figure 4. *In vitro–in vivo* correlation of drug ratio dependency as illustrated by CPX-351, a dual-drug liposome of cytarabine and daunorubicin. **(A)** A molar ratio of 5:1 cytarabine:daunorubicin provides synergistic leukemia cell killing *in vitro*. **(B)** In *in vivo* studies using the P388 murine leukemia model, BDF-1 mice received intravenous treatment on days 1, 4 and 7 with saline or a dual-drug liposome of cytarabine and daunorubicin at molar drug ratios. Formulations were dosed at their MTD with the exception of the 5:1 molar drug ratio (i.e., CPX-351), which was dosed at 0.8 of its MTD. The day 55 survival rate was highest with the 5:1 cytarabine:daunorubicin molar drug ratio. CYT: Cytarabine; DN: Daunorubicin; MTD: Maximum-tolerated dose; N/A: Not applicable. Reproduced with permission from [24] © Elsevier (2009).

Table 2. Overview of the clinical program for CPX-351 in acute myeloid leukemia.

Trial	Patients	Main objectives	n	Main results	Ref.
Phase I, study 101 NCT00389428	Relapsed or refractory AML or ALL, high-risk MDS	Identify MTD; evaluate persistence of 5:1 CYT:DN molar ratio, assess safety, determine whether CR could be achieved	48	MTD = 101 units/m ² given on days 1, 3 and 5 of each induction course; 5:1 molar ratio was maintained for up to 24 hours; Efficacy: nine CRs, one CRp in 43 patients with AML; one CR in three patients with ALL DLTs: congestive heart failure, hypertensive crisis, persistent cytopenias beyond 56 days (in one patient each)	[31,32]
Phase II, study 204 NCT00788892	Newly diagnosed AML in elderly patients (age ≥60 and ≤75 years)	Compare CPX-351 with 7 + 3 regimen with respect to CR + CRi rate and duration, induction mortality, EFS, OS and HCT rate	126	Efficacy results for CPX-351 vs 7 + 3, respectively¹: CR + CRi: all patients, 66.7 vs 51.2% (p = 0.07); high-risk, 77.3 vs 38.5% (p = 0.03); sAML, 57.6 vs 31.6% (p = 0.06); Remission duration, median: all patients, 8.9 vs 8.6 months; EFS, median: all patients, 2.0 vs 6.5 months (p = 0.36); high-risk, 1.8 vs 5.7 months (p = 0.38); sAML, 1.3 vs 4.5 months (p = 0.08); OS, median: all patients, 12.9 vs 14.7 months (p = 0.61); high-risk, 7.6 vs 11.0 months (p = 0.41); sAML, 6.1 vs 12.1 months (p = 0.01); HCT: 16.5 vs 24.4%; Safety results for CPX-351 vs 7 + 3, respectively: Mortality: 30-day: all patients, 3.5 vs 7.3% (p = 0.053); high-risk, 5.8 vs 12.0%; sAML, 3.0 vs 15.8%; 60-day: all patients, 4.7 vs 14.6%; high-risk, 7.7 vs 24.0%; sAML, 6.1 vs 31.6%; Median time: to neutrophil recovery to ≥1000/μl: 36 vs 32 days; to platelet recovery to ≥100,000/μl: 37 vs 28 days; Infection-related deaths: 3.5 vs 7.3%; Common (>15%) grade ≥3 nonhematologic adverse events: febrile neutropenia, 63.5 vs 51.2%; bacteremia, 35.3 vs 19.5%; pneumonia, 15.3 vs 19.5%; hypokalemia, 15.3 vs 12.2%	[37]
Phase II, study 205 NCT00822094	First relapse in patients with AML (age ≥18 and ≤65 years)	Compare CPX-351 with investigator's choice of salvage therapy (control) with respect to CR + CRi rate and duration, induction mortality, EFS, OS and HCT rate	125	Efficacy results for CPX-351 vs control, respectively¹: CR + CRi: all patients, 49.4 vs 40.9%; poor-risk, 39.3 vs 27.6%; Remission duration, median: <6 months, 33.3 vs 36.4%; 7–18 months, 53.1 vs 45.5%; >18 months, 13.6 vs 18.2%; EFS, median: all patients, 4.0 vs 1.4 months (p = 0.08); poor-risk, 2.0 vs 1.2 months (p = 0.08); OS, median: all patients, 8.5 vs 6.3 months (p = 0.19); poor-risk, 6.6 vs 4.2 months (p = 0.02); HCT: all patients, 46.9 vs 47.7%; poor-risk, 41.1 vs 37.9% Safety results for CPX-351 vs control, respectively: Mortality: 30-day: all patients, 7.4 vs 4.5%; poor-risk, 8.9 vs 6.9%; 60-day: all patients, 14.8 vs 15.9%; poor-risk, 16.1 vs 24.1%; 90-day, all patients: 18.5 vs 29.5%; poor-risk: 21.4 vs 37.9%; Median time: to neutrophil recovery to ≥1000/μl, 42 vs 34 days; to platelet recovery to >100,000/μl, 45 vs 35 days; Infection-related deaths: 9.9 vs 9.1%; Common (>15%) grade ≥3 nonhematologic adverse events: febrile neutropenia 54 vs 34%; bacteremia, 30 vs 43%; pneumonia, 22 vs 9%	[38]
Phase III, study 301 NCT01696084	Older patients (60–75 years of age) with newly diagnosed, high-risk sAML	Compare CPX-351 with 7 + 3 regimen with respect to OS, EFS and HCT rate	309	Efficacy results for CPX-351 vs 7 + 3, respectively: EFS, median: 2.53 vs 1.31 months (HR = 0.74; one-sided p = 0.011); OS, median: 9.56 vs 5.95 months (HR = 0.69; one-sided p = 0.003); HCT: 34 vs 25% Safety results for CPX-351 vs 7 + 3, respectively: Median time: patients with CR + CRi, to neutrophil recovery to >500/μl, 35 vs 29 days (first induction), 35 vs 28 days (second induction); to platelet recovery to >50,000/μl, 36.5 vs 29 days (first induction), 35 vs 24 days (second induction); Common (>15%) grade ≥3 nonhematologic adverse events: febrile neutropenia, 68 vs 71%; pneumonia, 20 vs 15%; and hypoxia, 13 vs 15%	[39,40]

¹A p-value of 0.1 was used to indicate statistical significance [37,38].

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; CR: Complete remission; CRi: Morphologic complete remission with incomplete blood count recovery; CRp: Complete remission with incomplete platelet recovery; CYT: Cytarabine; DLT: Dose-limiting toxicity; DN: Daunorubicin; EFS: Event-free survival; HCT: Hematopoietic cell transplantation; HR: Hazard ratio; MDS: Myelodysplastic syndrome; MTD: Maximum-tolerated dose; OS: Overall survival; sAML: Secondary acute myeloid leukemia.

Figure 5. Kaplan–Meier survival curves for older patients with newly diagnosed secondary acute myeloid leukemia randomized to induction treatment with CPX-351 versus 7 + 3 in a Phase II clinical trial. (A) Overall survival. (B) Event-free survival.

sAML: Secondary acute myeloid leukemia.

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A second Phase II clinical trial (NCT00822094) evaluated CPX-351 versus investigator's choice of intensive chemotherapy in adults (19–65 years of age) with first-relapse AML who had an initial complete response lasting ≥ 1 month (Table 2) [38]. Among a subset of patients with poor-risk first-relapse AML, median overall survival was 6.6 months for CPX-351-treated patients compared with 4.2 months for those receiving salvage chemotherapy

(hazard ratio = 0.55; $p = 0.02$) [38]. Higher response rates, lower 60-day mortality and longer event-free survival were also observed with CPX-351 compared with salvage chemotherapy treatment among poor-risk patients [38].

A Phase III, randomized, controlled trial (NCT01696084) evaluated CPX-351 versus 7 + 3 induction in older patients (60–75 years of age) with newly diagnosed, high-risk sAML (Table 2) [39]. For initial induction therapy, CPX-351 was dosed at 100 units/m² (100 mg/m² cytarabine and 44 mg/m² daunorubicin) on days 1, 3 and 5, with reinduction on days 1 and 3; for consolidation, CPX-351 was dosed at 65 units/m² (65 mg/m² cytarabine and 29 mg/m² daunorubicin) on days 1 and 3. Standard dosing was used for 7 + 3 induction and consolidation. Up to two inductions and two consolidations were permitted [39]. CPX-351 demonstrated a substantial improvement in outcomes compared with 7 + 3, including an increased overall response rate and prolonged median overall survival (9.56 vs 5.95 months; hazard ratio = 0.69; one-sided $p = 0.003$) [39,40]. Additionally, a greater proportion of patients in the CPX-351 cohort underwent allogeneic hematopoietic cell transplantation compared with 7 + 3 (34.0 vs 25.0%) [39].

Another *post hoc* exploratory analysis evaluated outcomes in patients with baseline *FLT3* mutations enrolled in the Phase III clinical trial (CPX-351, $n = 22$; 7 + 3, $n = 22$) [44]. Among patients with *FLT3* mutations, response rates were 68.2% with CPX-351 versus 25.0% with 7 + 3, and median overall survival was 10.25 versus 4.55 months, respectively [44]. The finding of notable efficacy in patients with the *FLT3* mutation was consistent with the results of a study that examined the *ex vivo* cytotoxic activity of CPX-351 against a wide range of blast types that were harvested from patients with hematologic malignancies [45]. That study demonstrated that CPX-351-treated leukemia blasts displaying the internal tandem duplication in the juxtamembrane domain of *FLT3* (*FLT3*-ITD) showed substantially lower mean IC₅₀ values (0.29:0.058 μ M) compared with *FLT3*-ITD-negative blast samples (1.32:0.26 μ M; $p = 0.047$ for comparison) [45].

Across studies, the safety profile of CPX-351 was similar to that of conventional 7 + 3 chemotherapy (Table 2), which is consistent with the development of CPX-351 being driven by the identification of the synergistic drug ratio to achieve effective antitumor activity rather than simply making the drugs less toxic [37–39]. Across studies, the most frequently reported adverse events for CPX-351 were febrile neutropenia, bacteremia, pneumonia and hypoxia (Table 2) [37–39]. CPX-351 was associated with an acceptable safety profile due to reduced drug exposure to healthy tissues, resulting from a lower administered amount of drug versus 7 + 3, sequestering of drug within the liposome, and internalization of the liposomes by leukemia cells to a greater extent than normal bone marrow cells [26]. Patients treated with CPX-351 had slower neutrophil recovery and platelet recovery compared with the control arm [37–39], which may be related to the greater and more prolonged CPX-351 drug exposure in the bone marrow [26,33]. Despite prolongation of myelosuppression, the rates of infection-related adverse events were low with both CPX-351 and control treatment [37,38].

CombiPlex drug combinations beyond CPX-351

Although CPX-351 is currently the most well studied CombiPlex agent, clinical data are also available for CPX-1, a combination of irinotecan and floxuridine at a 1:1 ratio. In a Phase I, dose-escalation study of CPX-1 in patients with advanced solid tumors, the 1:1 drug ratio was maintained *in vivo* for 8–12 h [21]. Anticancer activity was observed, with 13% of patients with colorectal cancer reaching partial response and 73% achieving disease control. Outpatient administration of CPX-1 was generally well tolerated; the most frequently reported grade 3/4 adverse events were diarrhea, neutropenia and hypokalemia [21].

The CombiPlex platform was recently expanded to potentially include combinations of molecularly targeted agents, using a hydrophobic prodrug nanoparticle (HPN) technology [1,5,20]. A prodrug conjugate was synthesized for the MEK inhibitor selumetinib in combination with the Akt inhibitor ipatasertib using cholesterol as a hydrophobic anchor joined via hydrolysable linkers that regenerate the parent drugs upon cleavage [20]. These HPN-associated drugs displayed markedly increased plasma exposure, decreased toxicity and increased efficacy in preclinical tumor models [20]. Importantly, this CombiPlex formulation of molecularly targeted agents displayed drug ratio-dependent efficacy in preclinical tumor models, consistent with the observations made with traditional cytotoxic agent combinations [20].

Similarly, prodrug conjugates of HSP90 inhibitor, AUY922 and docetaxel were anchored to cholesterol via a diglycolate linker and co-formulated into HPN. In preclinical studies that compared the HPN-encapsulated drugs to free drug, the CombiPlex formulation demonstrated a prolonged plasma concentration, reduced toxicity and increased tumor growth inhibition in xenograft models [20].

Conclusion

Traditional combination regimens may be unable to capture the full potential of combination therapy because they are based on combining two or more individually developed drugs with uncoordinated pharmacokinetic and pharmacodynamic properties. The CombiPlex technology platform provides a means of improving drug combinations by identifying and preserving synergistic drug ratios and coordinating drug exposure to target tissues. CPX-351, a dual-drug liposomal encapsulation of cytarabine and daunorubicin delivered at a synergistic 5:1 molar ratio to leukemia cells, is the most advanced product that uses the CombiPlex platform. CPX-351 has demonstrated significantly improved outcomes compared with standard 7 + 3 cytarabine/daunorubicin chemotherapy in patients with high-risk AML. The identification and evaluation of synergistic combinations as early as possible in the development process, using technology platforms such as CombiPlex, may provide a path to enhance efficacy, improve safety and reduce the time and patient resources required to create effective combination therapies.

Future perspective

The number of novel agents for the treatment of cancers is rapidly expanding and, with them, the number of possible therapeutic drug combinations. A rational, prospective approach to identify and validate those combinations with the greatest potential to improve patient outcomes is needed. The CombiPlex platform provides such an approach and, thus, could be a valuable tool in assessing and refining potential drug combinations prior to clinical trials, thereby increasing the likelihood of clinical success. It is hoped that rational drug design would decrease the time required for a potential drug combination to go from the lab to the clinic and streamline the clinical evaluation process for drug combinations. It is likely that with nanoparticle technologies capable of enhancing targeted delivery to specific tumor types, a greater variety of drug combinations may be possible for the treatment of a broad spectrum of malignancies in the decades to come. With sustained delivery of synergistic drug combinations to tumor cells, the CombiPlex platform has the potential to change the way researchers and healthcare professionals think about and develop combination therapy regimens.

Financial & competing interests disclosure

AW Tolcher has participated in a scientific advisory board for Celator Pharmaceuticals. LD Mayer is an employee of Jazz Pharmaceuticals (and a former employee of Celator Pharmaceuticals, now a subsidiary of Jazz Pharmaceuticals), which is commercializing CPX-351 and, as such, has a financial interest in CPX-351. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Medical writing and editorial assistance for the development of this manuscript were provided by KJ Brooks of SciFluent Communications, and were financially supported by Jazz Pharmaceuticals, Inc.

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Executive summary

- Traditional drug combinations involve concurrent administration of separate drugs that were developed individually and may have discordant properties.

Challenges associated with current combination cancer therapy development

- Individual agents have different pharmacokinetics and pharmacodynamics that may prevent sustained delivery of a synergistic drug combination.
- Dosing of combination therapies is often based on the maximum-tolerated dose of the individual agents, rather than optimized activity and coordinated pharmacokinetics.
- Combination therapies frequently result in greater toxicity than either drug given individually, requiring that each agent be administered at reduced doses.

Improving combination therapy development processes

- The CombiPlex[®] platform identifies drug ratios with synergistic activity at the preclinical stage, making the development process more efficient, and may result in more effective drug combinations.
- Once a synergistic drug ratio is identified, nanoscale drug carriers are used to coordinate the pharmacokinetics and tissue exposure profiles of both agents, maintain the drug ratio over an extended period of time and preferentially deliver a synergistic drug ratio to tumor cells.

CPX-351: a CombiPlex-derived therapy for acute myeloid leukemia

- CPX-351, a CombiPlex proof-of-concept drug, is a dual-drug liposomal formulation of cytarabine and daunorubicin that maintains a synergistic drug ratio for over 24 h and is taken up to a greater extent by leukemia cells than by normal bone marrow cells.
- Compared with the conventional cytarabine and daunorubicin (7 + 3) regimen, CPX-351 treatment increases remission rates and prolongs overall survival in adults with high-risk or secondary acute myeloid leukemia.
- The safety profile of CPX-351 is consistent with that of conventional 7 + 3 therapy.

CombiPlex drug combinations beyond CPX-351

- Using a hydrophobic prodrug nanoparticle technology, the CombiPlex platform has the potential to include combinations of molecularly targeted agents.
- In preclinical models, hydrophobic prodrug nanoparticle-associated drugs demonstrate substantially increased plasma exposure, decreased toxicity and improved efficacy.

Conclusion

- The early identification and evaluation of synergistic drug combinations using technology platforms such as CombiPlex may provide a path to improve efficacy and/or safety and reduce the time and resources required to develop effective combination therapies.

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