Next Generation Chemotherapy: Life in the Old Dog Yet

Authors: Jess Hearn-Messenger, PhD, Senior Consultant
Mar Casajuana Ester, Consultant Intern
Anthony Walker, PhD, Managing Partner

Date of Publication: May 2023

alacrita
expertise-based consulting

Next Generation Chemotherapy: Life in the Old Dog Yet

In biotech and pharma, it's fair to say that cancer chemotherapy has fallen out of fashion, with interest shifting predominantly to targeted therapies and immunotherapy. Investors seem to shun chemotherapy projects as they, justifiably, believe that Big Pharma will be reluctant to engage, even when there is strong clinical data. However, chemotherapy remains a frontline treatment option for many patients, and malignancies like acute myeloid leukemia and pancreatic carcinoma still rely on it alone. And while it may not have the luster of a cutting-edge ATMP, we argue that there remains promise, and reward, in the technology, and that it shouldn't be so hastily dismissed by drug developers and investors.

This paper acknowledges the key concerns with chemotherapy but aims to showcase some of the emerging R&D strategies aimed at optimizing rather than replacing this vital treatment option.

Preview

Innovative next-generation approaches that optimize chemotherapy delivery:

- BioSight: exploits pharmacokinetics by covalently binding a cytarabine payload to asparagine which is inactive until cytarabine is gradually released at pharmacokinetics which decrease the systemic exposure.
- CytomX Therapeutics: antibody prodrug activated by proteases in the tumor microenvironment to reveal a targeting moiety against tumor markers coupled to the microtubule inhibitor DM4.
- Cairn Therapeutics: uses prodrug of a duocarmycin analogue which has selective intracellular release driven by an enzyme only found in the cytosol of cancer cells.
- Arjuna Therapeutics: Ag5 acts by amplifying the effect of tumor-produced ROS leading to cell death, while non-tumor cells remain unaffected.
- Transgene: oncolytic virus expressing a gene coding for cytosine deaminase that converts a prodrug into 5-FU.
- Processa Pharma: co-administers a novel enzyme inhibitor to prevent metabolism of 5-FU into an inactive, and potentially toxic, form.

Traditional chemotherapy

Traditional chemotherapy agents primarily affect either macromolecular synthesis and function of cancer cells by interfering with DNA, RNA, or protein synthesis or by affecting the appropriate functioning of the preformed molecule [1]. Sufficient interference with these cellular processes leads to cell cycle arrest and/or cell death, triggered by various mechanisms. Most chemotherapeutic agents can additionally promote tumor immunity by inducing immunogenic cell death (ICD) as part of its primary mode of action and can disrupt strategies that tumors use to evade the immune response [2].

Despite many of these compounds being decades old, they are still very much a part of modern-day cancer treatment and the only treatment option available in many hard-to-treat cancers. Bendamustine, an alkylating agent, is an excellent example of this, originally

developed in East Germany in the 1950s, it was "rediscovered" and introduced into the US in 2000s and has since become an integral part of many chemotherapy-based regimens for haematological malignancies. Chemotherapy is not only used in frontline treatment, but also in the neoadjuvant/adjuvant settings. One key benefit of chemotherapy, versus for example, immunotherapy, is the ability to assess responses to treatment much faster. Although predominantly generic, chemotherapy remains a billion-dollar market, while targeted and immunotherapies, take an overwhelming portion of the R&D focus.

Despite their prominence in the treatment landscape, there are clear pitfalls with existing chemotherapy drugs, which is leading to the emergence of next generation agents.

Chemotherapy side-effects

The non-targeted nature of chemotherapy means that high levels of drug may be required to exert an effect on an aggressive cancer. Patients who receive a higher dose than is optimal may therefore experience more severe side effects, which means they are often unwilling to continue with treatment. This can automatically exclude elderly and more fragile patients from the frontline option. Side-effects of chemotherapy usually include immunosuppression and bone marrow suppression, gastrointestinal discomfort, anemia, fatigue, hair loss, secondary tumors, infertility, cognitive impairment, and organ damage [3]. In some cases, these can seriously reduce quality of life and even lead to death.

> Both targeted and immune therapies are not devoid of toxicity concerns. Often issues are only detected down the line.
>
> In contrast, toxicity to chemotherapy typically presents itself earlier. This may reduce the likelihood of regulators revoking accelerated approvals on the grounds of unexpected longer-term safety signals.

Chemotherapy induced nausea and vomiting (CINV) affects up to 40% of patients and is the most common adverse effect [4]. It has a detrimental impact on a patient's quality of life, treatment compliance, and overall healthcare cost [5]. Highly emetogenic chemotherapy regimens typically include high-dose cisplatin, carmustine, cyclophosphamide, dacarbazine, mechlorethamine, streptozocin, and combinations of anthracyclines and phosphamide [6]. Established anti-emetic drugs like 5-HT3 antagonists, corticosteroids, and NK1 receptor antagonists are often used. However, there are numerous unmet needs, such as optimizing control of non-acute forms of CINV and increasing adherence to guidelines.

Chemotherapy induced anemia (CIA) is relatively common, yet underdiagnosed given its pathogenic complexity. It can also negatively impact quality of life, prognosis and response to treatment, particularly when radiation therapy is planned [7]. Depending on the severity, management can include iron or blood transfusion, however, there is significant debate as to which of the treatments is best, particularly when considering alterations in iron metabolism that many patients experience. Optimized iron formulations are being developed to treat CIA patients with either absolute or functional iron deficiency, however, more novel treatments are also in the clinic. For example, Roxadustat

(FibroGen Inc.), a hypoxia-inducible factor prolyl hydroxylase inhibitor, promotes coordinated erythropoiesis through increasing endogenous erythropoietin, improving iron availability, and reducing hepcidin. Approved for the treatment of chronic kidney disease associated anemia, it is currently in Phase 2 trials for CIA.

Chemotherapy-induced neutropenia (CIN) is a potentially fatal and common complication [8]. Febrile neutropenia remains one of the most common and urgent treatment challenges. Currently, the standard treatment for CIN is the use of a granulocyte colony-stimulating factor (G-CSF) to attenuate white blood cell counts [9]. However, G-CSF use is expensive, has adverse effects and could even initiate or accelerate the development of myelodysplasia or acute myeloid leukemia. FDA recently approved Cosela (trilaciclib) as the first therapy in its class to reduce the frequency of chemotherapy-induced bone marrow suppression in adults receiving certain types of chemotherapy for extensive-stage small cell lung cancer.

Other chemotherapy side effects include mucositis, neurotoxicity, and gastrointestinal issues. Not to mention infusion reactions, which can be immune or nonimmune-mediated reactions with the cause related to the drug, the vehicle, or to patient-related risk factors [10].

Versus chemotherapy, targeted therapies have a different toxicity profile and look to reduce the side-effect profiles for anticancer treatment and indeed toxicity concerns are broadly reduced in comparison. However, targeted therapies are not absent of toxicity issues. For example, although recognized as a breakthrough treatment option for patients with ovarian and breast cancers, poly (ADP-ribose) polymerase (PARP) inhibitors suffer with cytopenia toxicities. Interestingly, despite having the same target, PARP inhibitors olaparib, niraparib, and rucaparib have varying toxicity profiles, and long-term adverse events, in particular, are key clinical priorities, with many patients requiring dose reductions [11], [12]. Even immunotherapy, generally considered a safe option (lymphodepletion aside), has its toxicity issues with widespread inflammation, e.g., 1-2% of patients taking checkpoint inhibitors can have life-threatening side effects [13].

Currently, the likelihood of experiencing quality-of-life-changing side-effects is inarguably higher when treated with conventional chemotherapy versus targeted options. However, there are established approaches that allow patients to experience the efficacy benefit of chemotherapy while alleviating toxicity concerns - and a number of promising ones in the pipeline.

Chemotherapy resistance

Resistance of cancer cells to chemotherapy is a leading cause of cancer-associated death [14]. Heterogeneity among patients and tumors, and the versatility of cancer to circumvent therapies make drug resistance challenging to manage [15]. Ovarian cancer provides a jarring example of chemotherapy resistance where 50%-70% of platin-treated ovarian cancers recur within 1 year after surgery and chemotherapy.

Chemoresistance is driven by genetic mutations in various proteins involved in cellular mechanisms such as cell cycle, apoptosis, and adhesion. In addition, expression of drug efflux transporters, an active DNA-repair capacity and a resistance to apoptosis, enhanced DNA repair, overexpression of anti-apoptotic genes, altered expression of drug-metabolizing enzymes and inactivation of apoptotic gene products are also involved in resistance. It can also be driven by altered drug absorption and subcellular distribution and decreased drug activation [16]. To add an additional layer of complexity, research has shown fluctuation in response of cancers cells to chemotherapy, moving between states of response and states of resistance. There is also a point of no return, where resistance is

established and can't be reversed [17]. Ongoing research aims to keep cancer cells in a state of response with an intact ability to activate apoptosis.

Better understanding of the mechanisms underlying the development of drug resistance is urgently needed and will facilitate developing novel therapeutic strategies and should lead to better clinical outcomes.

FAILURES AND SUCCESSES IN ADDRESSING CHEMOTHERAPY HURDLES

Drug Conjugates

The drug conjugate approach (which could also be considered a 'prodrug' approach) provides a molecular modification strategy that aims to optimize the physicochemical and pharmacological properties of chemotherapy drugs and by doing so improves targeting and pharmacokinetics, and decreases systemic toxicity [18]. The rational selection of the adequate pro-moiety and the type of linkage (e.g., ester, amide, carbamate, and phosphate) may determine the selectivity and toxicity.

There are several approved strategies successfully achieving this in the clinic, the most prominent being antibody-drug conjugates (ADCs). ADCs are designed to provide the targeting moiety of an antibody with the potent anticancer activity of a chemotherapy payload. The target antigen expressed on tumor cells is the navigator for ADC drugs to identify tumor cells and it also determines the mechanism (e.g., endocytosis) for the delivery of cytotoxic payloads into cancer cells. (Read: alacrita.com/blog/the-rise-and-rise-of-antibody-drug-conjugates)

> Antibody-drug conjugates (ADCs) have shown overwhelming commercial success in improving chemotherapy selectivity.

Since the first ADC, Mylotarg (gemtuzumab ozogamicin), was approved by the FDA in 2000, there have been 15 ADCs approved worldwide [19]. Most recently, in November 2022, the FDA approved ImmunoGen's Elahere (mirvetuximab soravtansine-gynx) for folate receptor α (FRα)–positive platinum-resistant ovarian cancer. It also approved the VENTANA FOLR1 (FOLR-2.1) RxDx Assay (Ventana Medical Systems, Inc.) as a companion diagnostic device to select patients for the above indication [20].

This indication had previously been entirely reliant on chemotherapy; Elahere is a first-in-class ADC linked to the maytansinoid payload DM4, a potent tubulin-targeting agent. Its accelerated approval was based on efficacy outcomes from the SORAYA study measuring investigator-assessed overall response rate (ORR) and duration of response (DOR). In the efficacy evaluable population of patients who had platinum-resistant, measurable disease, and received at least one dose (104 patients), the confirmed ORR was 31.7% and median DOR was 6.9 months.

There is an expanding field for delivering cytotoxic payloads, that now includes peptide drug conjugates (PDC), small molecule-drug conjugates (SMDC), immune-stimulating antibody conjugates (ISAC), antibody-oligonucleotide conjugates (AOC), radionuclide drug conjugates (RDC), antibody fragment-drug conjugates (FDC), aptamer drug conjugates (ApDC), antibody cell drug conjugates (ACC), antibody degraducer conjugates (ADeC) and virus-like drug conjugates (VDC).

Aura Biosciences is developing VDC technology which uses virus-like particles (VLP) derived from the human papillomavirus (HPV) that bind specifically to tumor-modified heparan sulfate proteoglycans and not to normal cells. These VLPs can be loaded or conjugated with chemotherapy drugs [21]. The company's lead is belzupacap sarotalocan (AU-011) which actually combines its VDC technology with photodynamic therapy (PDT), where the VLP is conjugated to a photosensitizer; this is currently in Phase 2 for choroidal melanoma.

Prodrugs

Prodrugs are bio-reversible, inactive drug derivatives, which can convert into a parent drug in the body [22]. Traditional prodrug approaches aim to improve physicochemical and/or biopharmaceutical drug properties, however, modern prodrugs also include cellular and molecular parameters to accomplish desired drug effect and site-specificity.

Directed enzyme prodrug therapy (DEPT) was first envisaged in the 1970s and uses enzymes artificially introduced into the body to convert prodrugs into an active form in a desired location. Targeted enzyme/prodrug strategies have been investigated to improve tumor selectivity with reduced side effects [23]. The enzyme, or its encoding gene, is first delivered to the tumor site using a targeting carrier, and after clearance of the enzyme from circulation, the prodrug is administered and then converted to an active anticancer drug, thus achieving enhanced anticancer efficacy with decreased systemic toxicity. Unfortunately, these prodrug strategies have not been translated to practical clinical applications due to low stability of bioactive carriers, scarcity and heterogeneity of tumor-specific antigens, poor delivery efficiency, immunogenicity of the carrier, and pharmacokinetic characteristics.

A recent effort to target the enzyme more efficiently to tumor sites using magnetically directed enzyme/prodrug therapy (MDEPT) has shown some promising preclinical results [23]. Here, β-glucosidase (β-Glu) was targeted to tumors using magnetic nanoparticles, where amygdalin was activated and killed prostate cancer cells. However, toxicity concerns highlighted a need to inject amygdalin at the tumor site, rather than deliver systemically, which is clearly a limitation.

There are various, more common branches of this approach, including antibody-directed enzyme prodrug therapy (ADEPT), virus-directed enzyme prodrug (VDEPT) and gene-directed enzyme prodrug therapy (GDEPT).

ADEPT was initially developed to overcome limitations of ADCs as it did not need internalisation, but aimed to generate drug in the extracellular areas of the tumor. However, there are only a handful of ADEPT programs currently in the clinic [24]. The ADEPT concept has been translated into clinical application, with the carboxypeptidase G2 (CPG2) system perhaps the most notable. However, there was a clear immunogenicity issue with using this enzyme, in addition to inadequate tumor localisation [25], [26]. Recent research has tried to optimize this system, producing PEGylated-CNGRC-CPG2 fusion proteins, which retained bioactivity with lower immunogenicity. However, this is yet to be clinically tested [27].

There is a pipeline of next generation prodrug approaches that provide exciting reinventions of conventional chemotherapy with improved efficacy and toxicity profiles.

Looking at ongoing clinical programs, CytomX Therapeutics is using a combination of ADEPT and ADC technology [28]. It is developing praluzatamab ravtansine (CX-2009) - a drug conjugate consisting of a recombinant antibody prodrug activated by proteases in the tumor microenvironment, to reveal a targeting moiety against the tumor markers (e.g., CD166 and CD71). This is coupled to the microtubule inhibitor DM4 for the treatment of advanced solid tumors. CytomX has two products already in Phase 2 clinical development and a host of Big Pharma partners. Another company exploiting the tumour microenvironment (TME) is Avacta, incorporating a substrate that is sensitive to cleavage by fibroblast activation protein (FAP) which is highly expressed in the tumour stroma by cancer associated fibroblasts. This allows active drug to be relased only within the TME; it's lead product AVA6000 is a tumour-activated form of doxorubicin and currently in Phase 1a studies [62].

There doesn't appear to be much industry activity with VDEPT technologies, however, GDEPT is perhaps the most studied approach and involves the expression of an enzyme within target cells that can activate a prodrug [29]. Various transgene/prodrug pairs have been discovered and tested. Yet, there are only a few programs in the clinic, with over 30 programs having failed or been discontinued (Tocagen being a notable failure). Lower transduction and transfection efficiency means that the extent of cell killing typically depends on the bystander effect. Numerous successful early-phase clinical trials provided some support for this approach, however, it has shown several limitations, mostly stemming from inefficient delivery of the transgene.

Regarding ongoing programs, GeoVax is using adenoviral gedeptin coupled with fludarabine prodrug in its GDEPT product, where gedeptin codes for an enzyme derived from E. coli, called purine nucleoside phosphorylase (PNP). When fludarabine is taken up by cells previously treated with gedeptin it is converted to fluoroadenine, a cytotoxic agent approved for several cancer indications. A cycle of gedeptin therapy involves the intratumoral (IT) administration of three doses of gedeptin over a two-day period followed by the IV administration of fludarabine, once a day, for 3 days. Clearly, IT administration is not ideal. The program is currently in Phase 1/2 for head and neck cancers.

Transgene is developing TG6002, which is a recombinant oncolytic virus based on vaccinia virus Copenhagen strain expressing the FCU1 gene, which codes for a cytosine deaminase and that converts the prodrug 5-fluorocytosine (5-FC), when delivered orally, into 5-FU [30]. This allows the drug to target the cancer cell on multiple fronts, not just through the GDEPT mechanism, but also through immunogenic cell death. It is currently in clinical studies for GI cancers.

There are some technologies exploiting a more natural DEPT approach, for example utilizing endogenously expressed enzymes as drug-activators. For example, CellAct has developed CAP7.1, a prodrug of etoposide (topoisomerase II inhibitor), which is converted by carboxylesterases naturally found in the liver, gallbladder, and gastrointestinal tract tissues, into etoposide. It completed a randomized, multicentre clinical Phase 2 study for the treatment of biliary tract cancers (before it was licensed to Mundipharma for this indication) and showed promising results [31]. Disease control rate (DCR) was better for CAP7.1 vs. SoC (50% vs. 20%), with disease progression at 40% vs. 70%, respectively. Significantly longer median progression free survival (PFS) was achieved for CAP7.1 vs. SoC at 66 vs. 39 days, respectively.

Cairn Therapeutics is taking a similar approach, where its compound CT-262 is a prodrug of an analogue of duocarmycin which has selective intracellular release driven by an enzyme only found in the cytosol of cancer cells and that does not appear to be present in

healthy cells [32]. Results in animal models and organoids have been promising. Additionally, the company has shown that the activated prodrug is not a substrate for membrane glycoproteins and is therefore unlikely to be subject to efflux mechanisms of resistance. Its stable interaction with DNA may also prevent repair mechanisms from causing drug resistance.

Processa Pharma is taking another interesting approach to actively inhibit the effect of endogenous enzymes on prodrugs, by exploiting the existing prodrug of 5-FU, (capecitabine) which is a widely-used chemotherapy agent [33]. Currently ~80% of 5-FU is further metabolized by dihydropyrimidine dehydrogenase (DPD) to a non-active (and potentially toxic) product form, while only 20% of 5-FU is metabolized through successive phosphorylation steps to facilitate anticancer activity. The program therefore co-administers PCS6422, which is an irreversible inhibitor of DPD, with capecitabine to allow more 5-FU to be metabolized into active 5-FU nucleotides. Processa reported that a single dose of PSC6422 in a Phase 1b trial in GI cancers successfully inhibited DPD, resulting in capecitabine being approximately 50 times more potent (full results not published). A Phase 2b is ongoing and the company has several other similar programs.

Another prodrug approach, taken by Biosight, is to exploit pharmacokinetics of binding partners to drive activated drug release. It has covalently bound a cytarabine payload to asparagine in its lead asset BST-236, which is inactive in its intact prodrug form until cytarabine is gradually released at pharmacokinetics, which decrease the systemic exposure to peak toxic cytarabine levels. This results in reduced systemic toxicity and relative sparing of normal tissues, enabling therapy with high cytarabine doses to patients otherwise unfit to receive it. A Phase 1/2 evaluated BST-236 in older or unfit-for-intensive-therapy (including HDAC, which remains fronline SoC) patients with acute leukemia. ORR was 29.6%; however, a subgroup analysis of newly diagnosed patients with AML, de novo or secondary to myelodysplastic syndrome, unfit for standard induction (median age 78), demonstrated ORR of 45.5% [34]. Patients experienced only mild side effects and had complete hematological recovery within 1 month.

Other prodrug approaches can exploit the natural cancer environment, e.g., reduced pH, elevated ROS or glutathione levels. Hypoxia is an important characteristic of most solid malignancies and is closely related to tumor prognosis and therapeutic resistance [35]. Hypoxia-activated prodrugs (HAPs) are bioreductive drugs that are selectively activated under hypoxic conditions and that can target the hypoxic regions of solid tumors. Despite many of these drugs reaching the clinic, efficacy and toxicity results have not been promising. Proposed solutions are primarily centered around testing combination approaches, developing biomarkers to identify likely responders, and reconsidering solid tumors as targets; micro-metastases, which are highly hypoxic and often chemoresistant, might be better targets.

ROS-inducing therapies

Cancer cells have higher levels of reactive oxygen species (ROS) than normal cells as a result of hypermetabolism, therefore this can be exploited to improve selectivity [36]. The acceleration of accumulative ROS disrupts redox homeostasis and causes severe damage in cancer cells. Pro-oxidative agents are not a modern research concept, indeed most conventional chemotherapy agents induce ROS. Therefore anticancer therapies that further induce oxidative stress by increasing ROS and/or inhibiting antioxidant processes have received significant attention, particularly those with mitochondrial-targeting. An example of an approach to inducing ROS in cancer treatment is that in development by Arjuna Therapeutics which is using so-called Therapeutic Molecular Clusters (TMC's) [37]. It's lead product, Ag5, acts as a catalyst inside cells to increase the oxidation of proteins

by ROS leading to cell death. A crucial advantage of this over previous ROS-dependent cancer killing therapies is that Ag5 does not induce ROS production, but only amplifyies the effect of tumor-produced ROS, meaning that non-tumor cells can remain unaffected. The company is still in preclinical development with this approach.

> Mitochondrial aberrations and ROS imbalance found in cancer cells are exploitable selectivity mechanisms for new chemotherapy approaches.

Photodynamic therapy (PDT) has been around for a long time with the phenomenon underlying its scientific basis discovered in the 1900s and has the aim of inducing ROS and selectively killing cancer cells. However, its widespread adoption has been constrained by limited accessibility of deep solid tumors to illumination [38]. Conversely, easily accessible tumors are the ones most amenable to PDT, but also the most amendable to surgery. In PDT, a light-activatable compound is merged with a nanocarrier agent which accumulates in tumors through the enhanced permeability and retention (EPR) effect (or with high affinity ligands such as peptides, antibodies and nucleic acids), targeting the cancer cell [39]. When exposed to a predetermined wavelength the photosensitizer (PS) generates ROS, which is assimilated by the tumor, killing it, and triggering an immune response causing the surrounding cancer cells to induce apoptosis.

Only a handful of PDT therapies have been approved, most of which are for oncology indications, although it is also used for actinic keratosis, and in some countries for age-related macular degeneration [40]. Efficacy can be limited due to poor pharmacokinetic (PK) properties and tumor targeting; of course, attaching targeting moieties like antibodies and peptides to carriers can also boost targeting. There are even methods in early development aimed at targeting ROS production to specific organelles within the cell, for example the nucleus and mitochondria, where it can do the most damage [41], [42].

Perhaps more interesting, is an approach aimed at overcoming the clear hurdle of hypoxia in solid tumors, which limits PDT. A multi-step approach was proposed by Akkaya et al where photosensitization is carried out ex situ in the presence of a 2-pyridone derivative and a PS. The endoperoxide product (storage compound) is then transferred to biological conditions and once triggered by hypoxia is bioreductively changed into a more labile version of itself. This specifically generates singlet oxygen without depending on, or depleting already low, tissue oxygen levels in tumors [43].

Photochemical internalization (PCI) is a further application of PDT, where photosensitizing molecules are trapped in endosomes along with macromolecules or chemotherapy drugs. Photoactivation of the PS disrupts the endosomal membranes so that chemotherapy molecules are released from endosomes inside cells and can reach their therapeutic target in the cell cytosol or nucleus. Compared with PDT, the main cytotoxic effect with PCI is disruption of the endosomal membrane resulting in delivery of the chemotherapy drug, and not to the photochemical reactions per se. Recent results using this approach with gemcitabine in patients with inoperable perihilar cholangiocarcinoma were promising, with an ORR of up to 60% [44].

MAKING THE MOST OF EXISTING THERAPIES

There are clearly some exciting next generation chemotherapy products in development, however, there are also established and pipeline approaches to optimizing, rather than replacing, current conventional chemotherapy use.

Optimized dosing

Dosing chemotherapeutic drugs on either a body surface area (BSA) or a weight basis fails to account for pharmacokinetic differences between patients. These are caused by inter-individual variability in drug absorption, distribution, metabolism and excretion rates, which can essentially lead to inadequate drug exposure levels [45]. The International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) evaluated clinical evidence of patients with colorectal or head-and-neck cancer receiving common 5-FU regimens and stated that only ~25% of patients experience exposure within the therapeutic window [46].

> Dose delivery optimization strategies are a fast and economic solution to optimizing conventional chemotherapy treatment.

In Clinical trials where 5-FU dosing was adjusted, a significantly improved ORR resulted, along with a trend to higher survival rate, and fewer toxicities [47]. Less successful was a Phase 3 trial of paclitaxel dosing, which did not result in enhanced treatment effectiveness, but did improve the benefit-risk profile. Interestingly, paclitaxel dosing can be exploited to achieve two different mechanisms for antitumor activity, where it is cytotoxic at higher dose levels and anti-angiogenic at lower, making it more versatile.

The efficacy of chemotherapeutics could also be increased by timing of dosing, for example, by coordinating with cell cycles. A Phase 2 clinical trial with tailored timings showed reduced toxicity and median time to treatment failure. However, interpatient differences in circadian phase, in addition to having multiple blood samples taken at different time points, would be a clinical barrier. Although still in its infancy, yet possibly offering a solution, preclinical studies have used metabolomics and transcriptomics combined with machine learning to determine a patient's circadian phase [48].

Pressurized intraperitoneal aerosol chemotherapy (PIPAC) is a recent technique for delivering chemotherapy that can be used in combination with systemic chemotherapy. It has shown promising results for patients with peritoneal metastasis (PM), which typically occurs in patients with gastric, ovarian, colorectal, appendix and pancreas cancer. This technique optimizes drug distribution homogeneity by applying an aerosol instead of a liquid. The increased temperature and intraperitoneal pressure counteract elevated intratumoral interstitial fluid pressure and can enhance the effects of chemotherapy [49]. A Phase 2 study showed high-grade tumor regression (TRG 3) and even complete tumor regression (TRG 4) in 39% and 10% of subjects, respectively. However, progress has been slow and the surgical device used to perform PIPAC procedure is commercialized by a single manufacturer [50].

Conjugation and nanoparticle encapsulation

Nanotechnology has developed rapidly in recent years, and nanoscale materials have unique physical, chemical, and biological properties. Nanoparticles usually have a small

particle size with a diameter within 10-200 nm and a large surface area to volume ratio, which allows them to adsorb and contain anticancer agents. The application of nanotechnology in tumor chemotherapy can increase the specificity of anticancer agents, increase the killing effect on tumors, while reducing toxic side effects [3], [51]. The enhanced permeability and retention (EPR) effect, which describes a universal mechanism in which macromolecular compounds like nanoparticles, liposomes and other polymer-conjugated drugs, means these structures can progressively accumulate in the tumor vascularized area and thus achieve targeting delivery and retention of anticancer compounds into solid tumor tissue [52].

There are plenty of nanomedicine chemotherapy drug formulations on the market. For example, Doxil was the first approved by the FDA in 1995 and is a PEGylated liposomal formulation of doxorubicin, which reduced cardiotoxicity versus free drug [53]. Another example is Abraxane, approved in 2005 for breast cancer, non-small cell lung cancer and pancreatic cancer as an albumin-bound paclitaxel (NP) which allowed for higher dosing and fewer side effects [54].

In addition to passive targeting, NPs can be modified to be more selective for cancer cells through active targeting where specific ligands recognised by cells at the tumor site are coupled to the surface of the NPs. The interaction between ligands on NPs and the receptors on the surface of cancer cells induces receptor-mediated endocytosis, which allows internalized NPs to successfully release therapeutic drugs. Some common ligands include folate, transferrin, hyaluronic acid and EGFR [55].

Additionally, nanoparticles can be designed to only release their contents under cancer-specific conditions, e.g., at acidic pH levels. This concept was demonstrated in vivo in breast cancer models, showing an improved release of doxorubicin from nanoparticles [56]. NPs targeting resistance mechanisms like overexpression of drug efflux transporters, defective apoptotic pathways, and hypoxic environment can lead to an improvement in the reversal of multidrug resistance. Although this research is still in early development.

Drug-device combinations

Local implantable drug delivery systems (IDDS) can be used as effective adjunctive therapy for solid tumors following thermal ablation for destroying the residual cancer cells and preventing tumor recurrence [57].

Dual-release implants have been clinically approved and currently used for cancer treatments, e.g., Gliadel polymer implants for treating malignant glioma. The implant aims to release the chemotherapy drug, carmustine, at the cancer target site over two phases; burst and sustained release. Gliadel wafers are placed on the surface of the resected tumor beds in recurrent tumors after initial resection [58]. In registrational clinical trials, overall survival was improved with Gliadel compared to placebo (13.9 vs. 11.6 months) for high grade gliomas [59]. Unfortunately, Gliadel wafers are also complicated by a wide range of side effects, including convulsions and cerebral edema [60].

Another example is the intravesical delivery system, TAR-200, used to deliver gemcitabine to patients with advanced muscle-invasive bladder cancer who are medically unfit for standard treatment [61]. The device combination is implanted into the bladder, where it can provide a continuous, low-dose, local delivery of gemcitabine. A recent Phase 1 study demonstrated an ORR of 40% showing beneficial preliminary efficacy in this elderly and frail cohort with limited treatment options.

FUTURE PERSPECTIVES FOR CHEMOTHERAPY

Increasing understanding of cancer progression and resistance mechanisms, as well as continuous development of innovative drug delivery systems and personalized medicine, make the outlook for chemotherapy promising. Despite this, in our experience, investor interest is not consistent when it comes to reinventing the chemotherapy wheel. However, the extensive clinical experience, coupled with the huge (and growing) market size should speak for itself.

> Chemotherapy may not be considered on-trend. However, the market opportunity for reinvention is significant and there are exciting companies struggling to attract funding. Not taking advantage of this is shortsighted in our view.

There is a recent spate of "voluntary" targeted drug withdrawals owed to lack of confirmed efficacy due to FDA retrenchment on its previous attitude to accelerated approvals. Chemotherapy agents are likely a much better bet against such regulatory revisionism. An example is last year's withdrawal of the PI3 kinase inhibitor umbralisib in non-Hodgkin lymphoma due to concerns over sudden death, despite initially being developed to be a safer PI3K inhibitor. With chemotherapy by contrast there isn't the same liability for problematic or preclusive toxicity emerging much further down the road.

Newer chemotherapy technologies will surpass the failures of first generation DEPTs. Passive prodrug approaches exploiting endogenous cancer environments, may offer a good compromise, and leans on our developing understanding of the importance of the tumor microenvironment.

The fastest route to embedding a new product into the clinic is to re-engineer an existing one. Chemotherapy is well-embedded into anticancer regimens, and despite its pitfalls, offers a quick, aggressive chance at tackling advancing cancers. VYXEOS - a fixed molar ratio of the long-established chemo agents daunorubicin and cytarabine for AML -, is an example of a successful, yet simplistic, re-engineering strategy. The success of ADCs is a testament to the opportunity for clinical and commercial returns for more innovative solutions using existing technologies.

Drug resistance is an undeniable hurdle in chemotherapy which isn't addressed by many of the next generation approaches. However, some (e.g., Cairn Therapeutics) have this risk at the forefront of its development program.

Clearly, longevity of effect and immune memory are important benefits of immunotherapies that chemotherapies cannot emulate (Read: alacrita.com/blog/cancer-vaccines). Although, the cost to achieving longevity can be high, for example, the bispecific blinatumomab has to be given as a continuous 28-day IV infusion for treating ALL.

Additionally, chemotherapy does not target cancer stem cells which are typically chemo and radiotherapy resistant. Combination regimens with next generation chemotherapies will be the key to tackling these concerns and over 30 immuno-chemotherapy combinations have been approved by the FDA already.

Chemotherapy remains a stalwart, frontline treatment for many cancers, though not without its challenges. The value in solving those challenges still presents a major opportunity, both to patients and to investors. As they say, "there's life in the old dog, yet.", and perhaps as an industry, it's worth more of our focus on its innovation.

REFERENCES

[1] A. Amjad, MT; Chidharla, A; Kasi, *Cancer Chemotherapy*. 2022.

[2] K. P. Fabian, B. Wolfson, and J. W. Hodge, "From Immunogenic Cell Death to Immunogenic Modulation: Select Chemotherapy Regimens Induce a Spectrum of Immune-Enhancing Activities in the Tumor Microenvironment," *Frontiers in Oncology*. 2021, doi: 10.3389/fonc.2021.728018.

[3] L. Yan, J. Shen, J. Wang, X. Yang, S. Dong, and S. Lu, "Nanoparticle-Based Drug Delivery System: A Patient-Friendly Chemotherapy for Oncology," *Dose-Response*. 2020, doi: 10.1177/1559325820936161.

[4] K. Gupta, R. Walton, and S. P. Kataria, "Chemotherapy-Induced Nausea and Vomiting: Pathogenesis, Recommendations, and New Trends," *Cancer Treatment and Research Communications*. 2021, doi: 10.1016/j.ctarc.2020.100278.

[5] A. K. Vaid *et al.*, "Expert Consensus on Effective Management of Chemotherapy-Induced Nausea and Vomiting: An Indian Perspective," *Front. Oncol.*, 2020, doi: 10.3389/fonc.2020.00400.

[6] Y. Razvi *et al.*, "ASCO, NCCN, MASCC/ESMO: a comparison of antiemetic guidelines for the treatment of chemotherapy-induced nausea and vomiting in adult patients," *Supportive Care in Cancer*. 2019, doi: 10.1007/s00520-018-4464-y.

[7] H. Abdel-Razeq and H. Hashem, "Recent update in the pathogenesis and treatment of chemotherapy and cancer induced anemia," *Critical Reviews in Oncology/Hematology*. 2020, doi: 10.1016/j.critrevonc.2019.102837.

[8] Y. Ba *et al.*, "Current management of chemotherapy-induced neutropenia in adults: key points and new challenges," *Cancer Biology and Medicine*. 2020, doi: 10.20892/j.issn.2095-3941.2020.0069.

[9] Y. Hashiguchi, M. Kasai, T. Fukuda, T. Ichimura, T. Yasui, and T. Sumi, "Chemotherapy-induced neutropenia and febrile neutropenia in patients with gynecologic malignancy," *Anticancer. Drugs*, 2015, doi: 10.1097/CAD.0000000000000279.

[10] A. McBride, "Infusion-related Reactions," *The oncology pharmacist*, 2010. .

[11] L. Willmott, "Toxicities With PARP Inhibitors for Ovarian Cancer," *Targeted Oncology*, 2021. .

[12] A. Chelariu-Raicu *et al.*, "PARP inhibitors: risk factors for toxicity and matching patients to the proper poly (ADP-ribose) polymerase inhibitor (PARPi) therapy," *Int. J. Gynecol. Cancer*, 2023, doi: 10.1136/ijgc-2022-003990.

[13] D. Carter, "Immunotherapy side effects: What to know," *MD Anderson*, 2018. .

[14] M. A. Desbats, I. Giacomini, T. Prayer-Galetti, and M. Montopoli, "Metabolic Plasticity in Chemotherapy Resistance," *Frontiers in Oncology*. 2020, doi: 10.3389/fonc.2020.00281.

[15] X. Wang, H. Zhang, and X. Chen, "Drug resistance and combating drug resistance in cancer," *Cancer Drug Resistance*. 2019, doi: 10.20517/cdr.2019.10.
[16] B. Mansoori, A. Mohammadi, S. Davudian, S. Shirjang, and B. Baradaran, "The different mechanisms of cancer drug resistance: A brief review," *Advanced Pharmaceutical Bulletin*. 2017, doi: 10.15171/apb.2017.041.
[17] J. F. Hastings *et al.*, "Memory of stochastic single-cell apoptotic signaling promotes chemoresistance in neuroblastoma," *Sci. Adv.*, 2023, doi: 10.1126/sciadv.abp8314.
[18] D. H. Jornada, G. F. Dos Santos Fernandes, D. E. Chiba, T. R. F. De Melo, J. L. Dos Santos, and M. C. Chung, "The prodrug approach: A successful tool for improving drug solubility," *Molecules*. 2016, doi: 10.3390/molecules21010042.
[19] Z. Fu, S. Li, S. Han, C. Shi, and Y. Zhang, "Antibody drug conjugate: the 'biological missile' for targeted cancer therapy," *Signal Transduction and Targeted Therapy*. 2022, doi: 10.1038/s41392-022-00947-7.
[20] FDA, "www.fda.gov/drugs," 2022. .
[21] R. C. Kines and J. T. Schiller, "Harnessing Human Papillomavirus' Natural Tropism to Target Tumors," *Viruses*. 2022, doi: 10.3390/v14081656.
[22] M. Markovic, S. Ben-Shabat, and A. Dahan, "Prodrugs for improved drug delivery: Lessons learned from recently developed and marketed products," *Pharmaceutics*. 2020, doi: 10.3390/pharmaceutics12111031.
[23] J. Zhou, J. Hou, J. Rao, C. Zhou, Y. Liu, and W. Gao, "Magnetically directed enzyme/prodrug prostate cancer therapy based on β-glucosidase/amygdalin," *Int. J. Nanomedicine*, 2020, doi: 10.2147/IJN.S242359.
[24] "Cortellis Intelligence Database," 2023.
[25] R. J. Francis *et al.*, "A phase I trial of antibody directed enzyme prodrug therapy (ADEPT) in patients with advanced colorectal carcinoma or other CEA producing tumors," *Br. J. Cancer*, 2002, doi: 10.1038/sj.bjc.6600517.
[26] S. K. Sharma and K. D. Bagshawe, "Translating antibody directed enzyme prodrug therapy (ADEPT) and prospects for combination," *Expert Opinion on Biological Therapy*. 2017, doi: 10.1080/14712598.2017.1247802.
[27] L. Al-mansoori, A. D. Al Qahtani, P. Elsinga, and S. K. Goda, "Production of Long-Acting CNGRC–CPG2 Fusion Proteins: New Derivatives to Overcome Drug Immunogenicity of Ligand-Directed Enzyme Prodrug Therapy for Targeted Cancer Treatment," *Technol. Cancer Res. Treat.*, 2021, doi: 10.1177/15330338211057371.
[28] "https://cytomx.com." .
[29] P. S. Kharkar and A. L. Jadhav, " Gene-Directed Enzyme–Prodrug Therapy (GDEPT) as a Suicide Gene Therapy Modality for Cancer Treatment ," 2022.
[30] "https://www.transgene.fr." .
[31] U. F. Pape *et al.*, "Effcacy and safety of CAP7.1 as second-line treatment for advanced biliary tract cancers: Data from a randomised phase ii study," *Cancers (Basel).*, 2020, doi: 10.3390/cancers12113149.
[32] "https://www.cairntherapeutics.com." .
[33] "https://www.processapharmaceuticals.com." .
[34] T. Zuckerman *et al.*, "BST-236, a novel cytarabine prodrug for patients with acute leukemia unfit for standard induction: A phase 1/2a study," *Blood Adv.*, 2019, doi: 10.1182/bloodadvances.2019000468.
[35] Y. Li, L. Zhao, and X. F. Li, "Targeting Hypoxia: Hypoxia-Activated Prodrugs in Cancer Therapy," *Front. Oncol.*, 2021, doi: 10.3389/fonc.2021.700407.
[36] S. J. Kim, H. S. Kim, and Y. R. Seo, "Understanding of ROS-Inducing Strategy in Anticancer Therapy," *Oxidative Medicine and Cellular Longevity*. 2019, doi: 10.1155/2019/5381692.
[37] "https://arjunatherapeutics.com." .
[38] M. R. Hamblin, "Photodynamic Therapy for Cancer: What's Past is Prologue,"

Photochemistry and Photobiology. 2020, doi: 10.1111/php.13190.
[39] G. Gunaydin, M. E. Gedik, and S. Ayan, "Photodynamic Therapy—Current Limitations and Novel Approaches," *Frontiers in Chemistry*. 2021, doi: 10.3389/fchem.2021.691697.
[40] Q. Xiao *et al.*, "Discovery and Development of Natural Products and their Derivatives as Photosensitizers for Photodynamic Therapy," *Curr. Med. Chem.*, 2017, doi: 10.2174/0929867324666170823143137.
[41] O. Karaman, T. Almammadov, M. Emre Gedik, G. Gunaydin, S. Kolemen, and G. Gunbas, "Mitochondria-Targeting Selenophene-Modified BODIPY-Based Photosensitizers for the Treatment of Hypoxic Cancer Cells," *ChemMedChem*, 2019, doi: 10.1002/cmdc.201900380.
[42] W. Chen *et al.*, " A C 5 N 2 Nanoparticle Based Direct Nucleus Delivery Platform for Synergistic Cancer Therapy ," *Angew. Chemie*, 2019, doi: 10.1002/ange.201900884.
[43] S. Ayan, G. Gunaydin, N. Yesilgul-Mehmetcik, M. E. Gedik, O. Seven, and E. U. Akkaya, "Proof-of-principle for two-stage photodynamic therapy: Hypoxia triggered release of singlet oxygen," *Chem. Commun.*, 2020, doi: 10.1039/d0cc06031c.
[44] J. Trojan *et al.*, "Photochemical Internalization of Gemcitabine Is Safe and Effective in Locally Advanced Inoperable Cholangiocarcinoma," *Oncologist*, 2022, doi: 10.1093/oncolo/oyab074.
[45] J. H. Beumer *et al.*, "Therapeutic Drug Monitoring in Oncology: IATDMCT Recommendations for 5-Fluorouracil Therapy," *Clin. Pharmacol. Ther.*, 2019.
[46] E. Gamelin *et al.*, "Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: Results of a multicenter randomized trial of patients with metastatic colorectal cancer," *J. Clin. Oncol.*, 2008, doi: 10.1200/JCO.2007.13.3934.
[47] L. DeRidder, D. A. Rubinson, R. Langer, and G. Traverso, "The past, present, and future of chemotherapy with a focus on individualization of drug dosing," *J. Control. Release*, 2022, doi: 10.1016/j.jconrel.2022.10.043.
[48] K. A. Dyar and K. L. Eckel-Mahan, "Circadian metabolomics in time and space," *Frontiers in Neuroscience*. 2017, doi: 10.3389/fnins.2017.00369.
[49] S. J. Tate and J. Torkington, "Pressurized intraperitoneal aerosol chemotherapy: a review of the introduction of a new surgical technology using the IDEAL framework," *BJS Open*. 2020, doi: 10.1002/bjs5.50257.
[50] S. Martellotto, C. Maillot, L. Villeneuve, C. Eveno, O. Sgarbura, and M. Pocard, "Restricted access to innovative surgical technique related to a specific training, is it ethical? Example of the PIPAC procedure. A systematic review and an experts survey," *International Journal of Surgery*. 2020, doi: 10.1016/j.ijsu.2020.07.004.
[51] Y. Yao *et al.*, "Nanoparticle-Based Drug Delivery in Cancer Therapy and Its Role in Overcoming Drug Resistance," *Frontiers in Molecular Biosciences*. 2020, doi: 10.3389/fmolb.2020.00193.
[52] J. Wu, "The enhanced permeability and retention (Epr) effect: The significance of the concept and methods to enhance its application," *Journal of Personalized Medicine*. 2021, doi: 10.3390/jpm11080771.
[53] O. M.E.R. *et al.*, "Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer," *Annals of Oncology*. 2004.
[54] J. Cortes and C. Saura, "Nanoparticle albumin-bound (nab™)-paclitaxel: improving efficacy and tolerability by targeted drug delivery in metastatic breast cancer," *Eur. J. Cancer, Suppl.*, 2010, doi: 10.1016/S1359-6349(10)70002-1.
[55] O. C. Farokhzad and R. Langer, "Impact of nanotechnology on drug delivery," *ACS*

Nano, 2009, doi: 10.1021/nn900002m.
[56] Q. Chen, X. Huang, G. Zhang, J. Li, Y. Liu, and X. Yan, "Novel targeted pH-responsive drug delivery systems based on PEGMA-modified bimetallic Prussian blue analogs for breast cancer chemotherapy," *RSC Adv.*, 2023, doi: 10.1039/d2ra06631a.
[57] M. Al-Zu'bi and A. Mohan, "Modelling of combination therapy using implantable anticancer drug delivery with thermal ablation in solid tumor," *Sci. Rep.*, 2020, doi: 10.1038/s41598-020-76123-0.
[58] J. Perry, "Gliadel Wafers in the Treatment of Malignant Glioma: A Systematic Review," *Curr. Oncol.*, vol. 14, no. 5, pp. 189–194, 2007.
[59] M. Westphal, Z. Ram, V. Riddle, D. Hilt, and E. Bortey, "Gliadel® wafer in initial surgery for malignant glioma: Long-term follow-up of a multicenter controlled trial," *Acta Neurochir. (Wien).*, 2006, doi: 10.1007/s00701-005-0707-z.
[60] E. L. Weber and E. A. Goebel, "Cerebral edema associated with Gliadel wafers: Two case studies," *Neuro. Oncol.*, 2005, doi: 10.1215/S1152851704000614.
[61] F. J. P. van Valenberg *et al.*, "Safety, tolerability, and preliminary efficacy of TAR-200 in patients with intermediate risk non–muscle-invasive bladder cancer: A phase 1 study.," *J. Clin. Oncol.*, 2023, doi: 10.1200/jco.2023.41.6_suppl.505.
[62] https://avacta.com/].

About Alacrita

Founded in 2009, Alacrita is an expertise-based consulting firm serving about 100 clients per year. We have a well-established track record of successful engagements, across a range of disciplines, therapeutic areas and technologies.

Our core team of partners has been carefully assembled with pharma and biotech executives who have deep domain knowledge and senior business acumen. Each brings at least 20 years of industry experience.

Alacrita's core team leverages a consulting group of over 250 functional specialists, allowing us to offer a versatile combination of depth and breadth in expertise that can be precisely tailored and adapted to our clients' needs. Our capabilities cover both the U.S. and Europe.

For more information on our expertise please visit www.alacrita.com.

London Office:

London BioScience Innovation Centre
2 Royal College St
London
NW1 0NH
United Kingdom

+44 207 691 4915
europe@alacrita.com

Boston Office:

Cambridge Innovation Center
One Broadway, Floor 14
Cambridge, MA
02142
United States

+1 617-714-9696
usa@alacrita.com