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The evolving role of DNA inter-strand crosslinks in chemotherapy

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Abstract

DNA crosslinking agents make up a broad class of chemotherapy agents that target rapidly dividing cancer cells by disrupting DNA synthesis. These drugs differ widely in both chemical structure and biological effect. In cells, crosslinking agents can form multiple types of DNA lesions with varying efficiencies. Inter-strand crosslinks (ICLs) are considered to be the most cytotoxic lesion, creating a covalent roadblock to replication and transcription. Despite over 50 years in the clinic, the use of crosslinking agents that specialize in the formation of ICLs remains limited, largely due to high toxicity in patients. Current ICL-based therapeutics have focused on late-stage and drug-resistant tumors, or localized treatments that limit exposure. In this article, we review the development of clinical crosslinking agents, our understanding of how cells respond to different lesions, and the potential to improve ICL-based chemotherapeutics in the future.

Keywords

chemotherapy; inter-strand crosslink (ICL); drug-resistance; DNA repair

Introduction

Crosslinking agents are a chemically diverse group of molecules that contain two or more reactive ends. In cells, the bi-functional nature of crosslinking agents can lead to covalent coupling between functional groups on DNA and other molecules. Reactions with DNA may involve one or both strands to form different types of lesions, including DNA mono-adducts, DNA-protein crosslinks, *intra*-strand crosslinks, and *inter*-strand crosslinks (ICLs) (Figure 1) [1]. Although crosslinking agents can produce a variety of DNA lesions in cells, cytotoxicity is often attributed to the formation of ICLs. By covalently coupling complementary strands of the DNA duplex, ICLs block strand separation that is required for fundamental cellular processes like DNA replication and transcription[2]. Failure to remove ICLs from DNA can block cell cycle progression and lead to cell death[3]. Defects in ICL repair can also lead to catastrophic chromosomal aberrations that promote tumorigenesis[4].

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Congenital defects in ICL repair are associated with two major cancer predisposition syndromes, Fanconi anemia (FA) and hereditary breast and ovarian cancer susceptibility (BRCA). Together, FA and BRCA proteins form an adaptive DNA damage signaling and repair pathway that promotes removal of ICLs and restores the damaged DNA[5, 6]. The FA and BRCA pathways are highly inter-connected and share extensive genetic overlap[7, 8]. While FA-deficient cells are particularly sensitive to ICLs[5], BRCA mutants are sensitive to a range of DNA lesions, including ICLs[9].

The high cellular toxicity of ICLs has been exploited clinically by numerous anti-cancer therapies. Cancer is typified by rapid, uncontrolled cell proliferation. Like many chemotherapeutics, crosslinking agents selectively target cancer by interfering with DNA synthesis[10]. Although some crosslinking agents are used extensively in the clinic, those that form ICLs with high efficiency are typically reserved for late-stage and drug-resistant tumors[11], or localized treatments that reduce potential side effects[12–15]. In this review, we provide an update on several studies that shed new light on how cells respond to different ICL-inducing agents and their potential for future therapeutic application.

Diversity of Clinical Crosslinking Agents

There are four major subgroups of crosslinking agents that have been developed for clinical use: nitrogen mustards, mitomycins, psoralens, and platinum-based compounds (Figure 2). Differences in chemical structure affect how these drugs interact with DNA and the type of lesions they create. The key features of each group are described below.

Nitrogen mustards

Nitrogen mustards are among the oldest and most extensively studied crosslinking agents[6, 16]. Several derivatives of nitrogen mustard (mechlorethamine) are still used regularly in the clinic, including melphalan and cyclophosphamide. Nitrogen mustards contain an N,N-bis-(2-chloroethyl) amine as the defining component. In cells, nitrogen mustards form mostly DNA mono-adducts and intra-strand crosslinks, with ICLs making up 5% or less of total lesions[16]. Nitrogen mustard ICLs are generated at the N7 of guanine in GpC (5’-G-phosphate-C-3’) or GpNpC DNA sequences[16]. Nitrogen mustard ICLs cause only limited DNA distortions, bending the duplex ~10 degrees and unwinding the helix by ~6 degrees[6].

Mitomycins

Mitomycins are a family of natural products that were originally isolated from *Streptomyces caespitosis* in the 1950s. Although several derivatives have been developed, Mitomycin C (MMC) remains the most clinically active. In cells, MMC can form up to 15% ICLs, in addition to a variety of DNA mono-adducts that make up over half of total lesions[6, 17]. Mitomycin C itself is only mildly reactive. In cells, MMC is “activated” by the reduction of its quinone moiety[11]. MMC ICLs are preferentially formed at CpG sequences[6, 11] and cause minimal distortion to the DNA helix[6, 18, 19].

Psoralens

Originally derived from plants, psoralen and derivatives like methoxypsoralen and trimethylpsoralen are photosensitive and become reactive when exposed to ultraviolet (UV) light[20]. Psoralens are planar in structure and able to intercalate between adjacent base pairs of duplex DNA. As a result, UV-activated psoralens readily form ICLs with high efficiency, ranging from ~40% with methoxypsoralen[21] to ~90% with trimethylpsoralen[6]. Psoralens preferentially react with TpA sequences in DNA to form covalent mono-adducts and ICLs. Psoralen ICLs do not bend the DNA duplex but do cause helical unwinding of ~25 degrees[6, 22, 23].

Platinums

A wide variety of platinum-based compounds have been developed for clinical use, including cisplatin, carboplatin, oxaliplatin, nedaplatin, and satraplatin. One of the most widely studied is cisplatin, which forms approximately 90% intra-strand crosslinks[24, 25] and only 1–2% ICLs in genomic DNA[26]. Cisplatin ICLs form almost exclusively at GpC sequences, reacting with the N7 of guanine[6]. Because cisplatin ICLs rest in the minor groove of DNA and force cytosine extrusion, these lesions cause severe distortion of DNA, bending the duplex ~47 degrees and unwinding the helix ~110 degrees[6].

Recognition and Repair of ICLs

The structure and position of an ICL play a major role in how the lesion is recognized and repaired in cells[27]. Various factors from different DNA repair pathways cooperate to recognize and remove ICLs from DNA, including those from nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination repair (HR), translesion synthesis (TLS), transcription coupled repair (TCR), and the FA/BRCA cancer predisposition pathways[6, 28]. Together, these pathways form an adaptive, non-linear stress response that coordinates removal of ICLs from DNA.

Replication-coupled repair

ICL repair occurs primarily during S phase after a replication fork collides with the crosslink[29, 30]. When replication forks are unable to bypass an ICL, a series of damage signaling events coordinate removal of the ICL, repair of the damaged DNA, and completion of DNA synthesis. For cisplatin ICLs, repair requires the convergence of two replication forks on the lesion[29]. In cells, the firing of dormant origins likely facilitates fork convergence when arrival of a neighboring fork is delayed[31]. Damage signaling pathways then promote dismantling of the replication machinery by the ubiquitin-selective p97 segregase, allowing repair enzymes to access the obstructed crosslink[32, 33]. Next, members of the FA/BRCA pathway, including FANCI-FANCD2[34], promote DNA incision by XPF-ERCC1 that “unhook” the ICL from one DNA strand[35, 36]. The resulting DNA double-strand break is repaired by homologous recombination[37] and the remaining ICL adduct is likely removed by NER.

ICL traverse

Replication forks have also been shown to “traverse” a trimethylpsoralen ICL, continuing DNA synthesis past the crosslink without repair[38]. This process is supported by FANCM, a DNA translocase that interacts with the replisome through the processivity factor PCNA[39]. Although the mechanism remains unclear, ICL traverse allows cells to complete DNA synthesis and repair the ICL at a later time, likely using a replication-independent mechanism described below.

Direct cleavage of psoralen ICLs

Unlike ICLs generated by other agents, psoralen crosslinks contain an N-glycosyl bond that can be cleaved directly by NER DNA glycosylases like NEIL1[40, 41] and NEIL3[42, 43]. Cleavage of the glycosidic bond does not require displacement of the replicative helicase and is not dependent on FANCI-FANCD2 or the associated nucleases[34, 42]. Thus, psoralen ICLs can be readily bypassed without the formation of a highly toxic DNA double-strand break. As with replication-coupled repair, the remaining ICL adducts are likely removed by NER.

Replication-independent repair

Outside of S phase, there are at least two distinct mechanisms for ICL recognition. First, global genome surveillance proteins like XPC-HR23B and DDB1-DDB2 are thought to recognize helical distortions caused by some ICLs[6, 44, 45]. Second, transcription of cross-linked DNA can lead to stalling of RNA polymerase[46, 47]. In both cases, subsequent repair is dependent on NER and TLS proteins[44, 46, 47]. After incisions unhook the ICL from one DNA strand, TLS polymerases fill in the single-stranded gap. The unhooked adduct is then removed from the other DNA strand by a second round of incisions and gap filling.

Targeting cancer with ICL therapeutics

Because of their potent anti-cancer properties, DNA crosslinking agents have remained at the frontline of chemotherapy for more than 50 years. There are currently over 4,400 open clinical trials that utilize crosslinking agents (Table 1). About a quarter of these are late-phase trials (phase III or IV) that have proven to be effective in early studies and show the most potential to improve the standard of care. Over half of all open crosslink trials utilize either cisplatin (~23%) or the nitrogen mustard derivative cyclophosphamide (~28%). However, clinical use of crosslinking agents that create ICLs with high efficiency remains limited due to high toxicity associated with treatment[48]. Together, MMC and psoralen compounds make up only 2% of open clinical trials, with the number of new registrations relatively unchanged over the last 12 years (Figure 3C). Thus, most crosslink-based treatments are not currently designed to create ICLs, but instead heavily favor the formation of other types of DNA lesions.

In the clinic, crosslinking agents are frequently used to induce cellular stress and enhance the cytotoxicity of other agents. In late-phase trials, over 95% of crosslink-based therapies involve combinations with other chemotherapeutic agents (Table 1). Combinational treatments typically administer drugs at lower doses than single-drug therapies, which can

reduce toxic side effects associated with treatment. Combining drugs that act through different mechanisms is also an established strategy for preventing the development of drug resistance, which remains one of the primary challenges for anti-cancer chemotherapies. Although many tumors are initially sensitive to treatment, cancer genomes are inherently unstable and can develop tolerance through several mechanisms. Cells can acquire both genetic and epigenetic changes that disrupt drug-target interactions, increase drug efflux from cells, and alter cellular signaling pathways that control the DNA damage response and cell death[49, 50]. On average, less than 12% of late-phase trials that utilize crosslinking agents target unresponsive, relapsed, or recurrent cancers, emphasizing the need to develop additional therapies that can treat drug-resistant tumors.

To combat both innate tolerance and acquired resistance to chemotherapeutics, many treatment strategies seek to exploit changes in cancer physiology. For example, cancer cells frequently acquire defects in DNA repair during both the development and progression of cancer[53]. Consequently, many tumors are highly sensitive to different types of DNA damage. In order to predict which tumors will be sensitive to treatment, a variety of biomarkers from different DNA repair pathways have been identified, including BRCA1, BRCA2, ATM, ATR, CHK1/2, and FANCD2[54–56]. Using patient-derived tumor samples, changes in gene sequence, protein expression, and post-translational modification have been shown to strongly correlate with cellular sensitivity to specific DNA damaging agents[57]. However, the impact that many biomarkers have in the clinic is limited by high analytical costs and a lack of viable treatment options. As a result, many treatments are administered without stratifying patients based on DNA repair capabilities.

Going forward, additional therapeutic strategies are needed to harness the potent cytotoxic effects of ICLs. Recent studies have revealed a diverse range of repair mechanisms utilized by cells in response to different ICL lesions. New roles have been identified for proteins like NEIL1/3, p97, and FANCM during ICL repair, highlighting these factors and their respective pathways as valuable targets for combinational therapy with crosslinking agents. With the advent of tumor profiling, novel molecular inhibitors, and a growing list of DNA damage response biomarkers, there is immense potential to exploit these mechanistic observations clinically. Establishing highly selective and targeted therapies will not only improve the efficacy of current ICL-based treatments, but also allow them to be applied more broadly by reducing the toxic side effects that serve as a barrier to widespread use.

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Highlights

- DNA crosslinking agents have been widely used in the clinic for over 50 years.
- Crosslinking agents can generate different DNA lesions with varying efficiencies.
- Due to high toxicity, most crosslink-based therapies avoid formation of ICLs.
- New mechanistic observations highlight promise for exploiting the potent effects of ICLs.

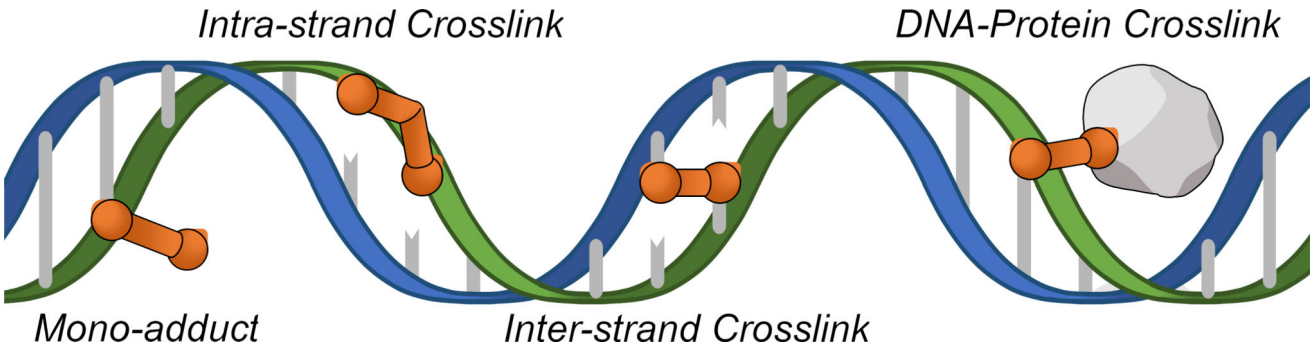

Figure 1. DNA lesions formed by crosslinking agents

Crosslinking agents are highly reactive molecules that can form multiple types of DNA lesions in cells.

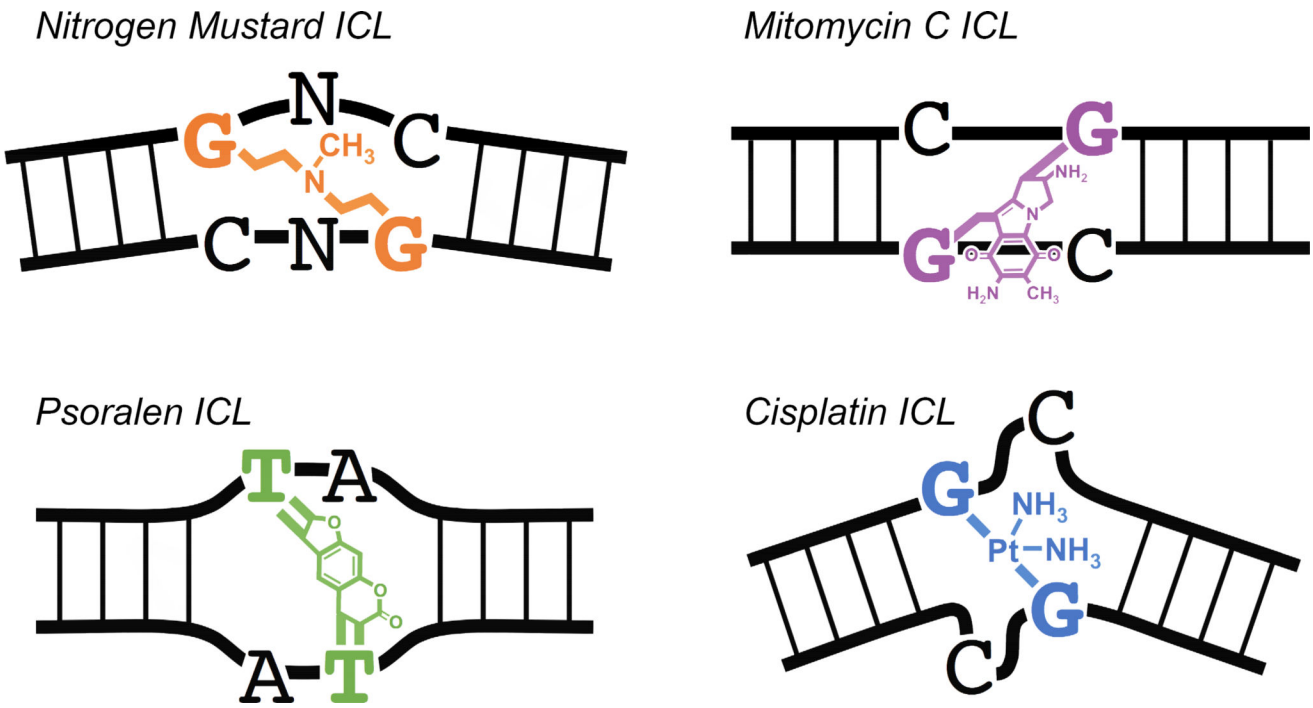

Figure 2. Diversity of ICL lesions

There are four major subgroups of DNA crosslinking agents. The ICLs produced by each group can vary widely in chemical structure and their effect on DNA topology. Nitrogen Mustard ICLs cause mild DNA bending and unwinding. Mitomycin C ICLs cause minimal distortion to DNA. Psoralen ICLs cause moderate DNA unwinding. Cisplatin ICLs cause severe DNA bending and unwinding.

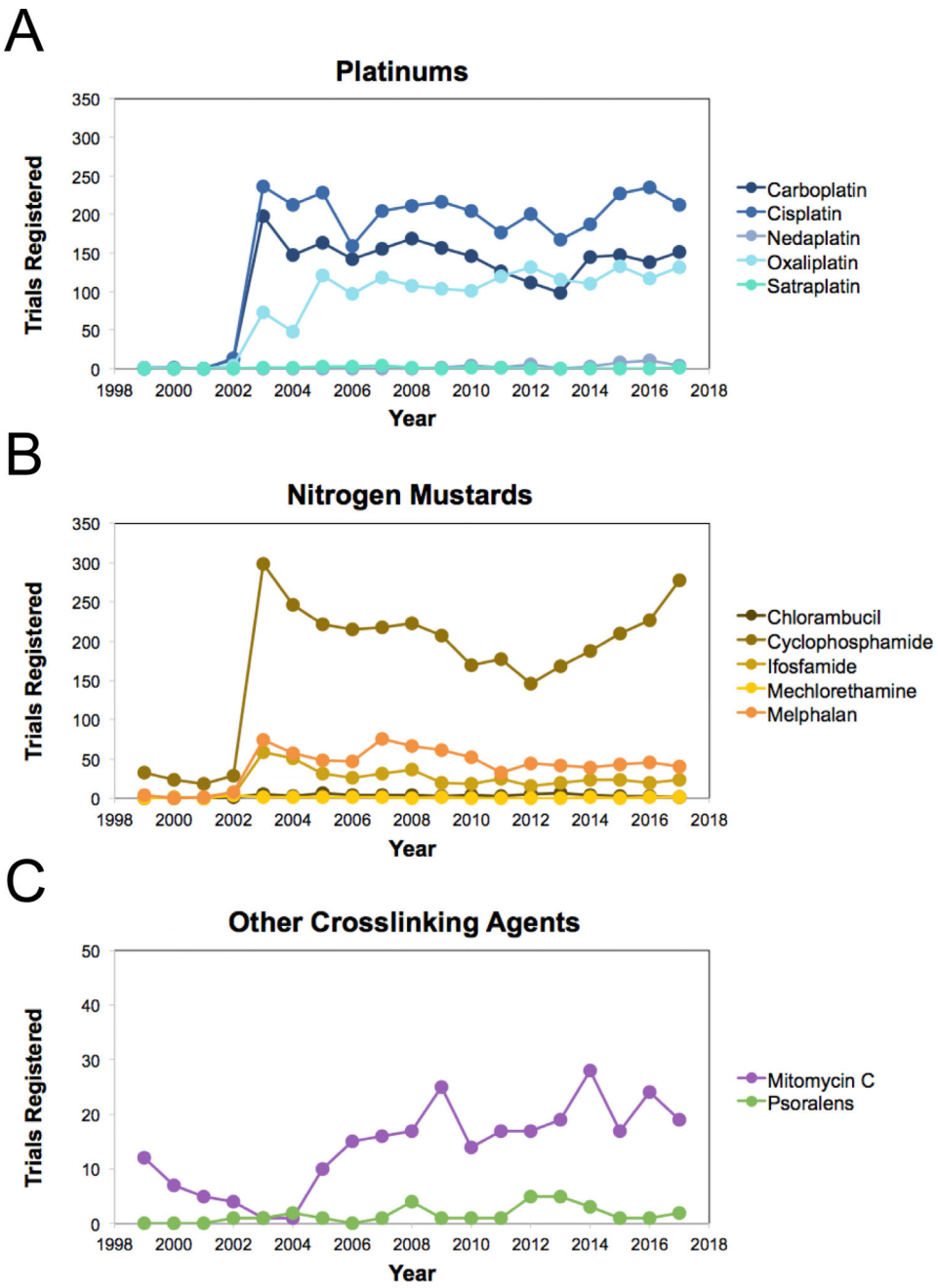

Figure 3. Clinical use of DNA crosslinking agents over time

The number of clinical trials registered each year with ClinicalTrials.gov is graphed for the most commonly used crosslinking agents from each major subgroup. ClinicalTrials.gov consists of privately and publicly funded clinical studies conducted around the world. The database was established by the Food and Drug Administration Modernization Act of 1997 (FDAMA) and is maintained by the National Library of Medicine (NLM) at the National Institutes of Health (NIH).

Table 1

Open clinical trials with DNA crosslinking agents. The number of open clinical trials registered with clinicaltrials.gov is listed for crosslinking agents from each major subgroup. ‘Total’ includes trials that are active and currently recruiting, enrolling by invitation, or active but not recruiting. ‘Late Phase’ is defined as phase III and phase IV trials. ‘Combo’ represents the number of late-phase trials that combine a DNA crosslinking agent with at least one additional chemotherapeutic agent. ‘Resistant’ represents the number of late-phase trials that target drug-resistant, refractory, relapsed, or recurrent cancers unresponsive to previous therapy. All data current through 01/ 01/2018.

		Late phase			
Name	**Total**	**Total**	**Combo**	**Resistant**	**Conditions treated**
Platinums					
Cisplatin	1035	315	299	29	Non-small cell lung cancer, hepatocellular carcinoma, biliary tract cancer, mesothelioma, breast cancer, brain neoplasms, and gynecological cancers
Carboplatin	748	197	197	44	Abdominal and rectal cancers, liver cancer, esophageal cancer, neuroblastoma, pancreatic cancer, biliary tract cancer, hepatocellular carcinoma, lung cancer, breast cancer, and gynecological cancers
Oxaliplatin	574	168	168	11	Digestive system cancer, liver cancer, esophageal cancer, neuroblastoma, pancreatic cancer, peritoneal carcinomatosis, bile duct cancer, biliary tract cancer, hepatocellular carcinoma, and lung cancer
Nedaplatin	18	6	6	–	Nasopharyngeal, esophageal cancer, and non-small cell lung cancer
Satraplatin	1	–	–	–	Prostate cancer
Nitrogen mustards					
Cyclophosphamide	1222	264	257	22	Crohn’s disease, neuroblastoma, sarcomas, breast cancer, endometrial cancer, leukemia, lymphoma, myeloma, thyroid cancer, systemic sclerosis, and lupus nephritis
Ifosfamide	428	30	28	4	Small cell lung cancer, soft tissue sarcoma, lymphoma, osteosarcoma, germ cell tumor, nasopharynx carcinoma, bladder cancer, testicular cancer, squamous cell carcinoma of the penis, cervical cancer, pleuropulmonary blastoma, neuroblastoma, unresectable sinonasal tumors, and pancreatic cancer
Melphalan	268	40	40	7	Metastatic melanoma, multiple myeloma, hematopoietic stem cell transplantation, prostate cancer, colorectal cancer, lymphomas, brain neoplasms, leukemia, retinoblastoma, bile duct cancer, spinal tumors, autoimmune disease, sickle cell disease, testicular cancer, myelofibrosis, Ewing’s sarcoma, and amyloidosis
Chlorambucil	18	9	7	1	Chronic lymphocytic leukemia, extranodal MALT lymphoma, small cell lymphoma, and bladder cancer
Mechlorethamine	9	2	2	–	Mycosis fungoides, cutaneous T-cell lymphoma, and Hodgkin’s lymphoma
Other					
Mitomycin C	89	30	13	5	Glaucoma, bladder cancer, colorectal cancer, anal cancer, pancreatic cancer, bile duct cancer, gastric cancer, liver cancer, head and neck cancers, peritoneal cancer, gynecological cancers, mesothelioma, gastrointestinal cancer, metastatic breast cancer, pterygium, presbyopia, corneal opacity, obstructive airway disease, and post-LASIK keratectasia
Psoralens	4	1	–	–	Vitiligo and cutaneous T-cell lymphoma