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Gemcitabine-based Chemoradiation in the Treatment of Locally Advanced Head and Neck Cancer: Systematic Review of Literature and Meta-analysis

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Abstract

Background: The non-surgical management of locoregionally advanced (LA) squamous cell carcinoma of the head and neck (SCCHN) has moved to a multimodality treatment approach. Concurrent chemoradiation (CCRT) has become the standard of care and is mostly (cis)platinum-based. Platinum-based CCRT improves locoregional control and overall survival when compared to radiotherapy alone, but this approach is hampered by significant toxicity. Therefore, alternative ways to enhance the radiation effects are worth investigating. Gemcitabine (2',2'-difluorodeoxycytidine), in addition to its activity against a variety of solid tumors, including SCCHN, is one of the most potent radiosensitizers, and has an overall favorable safety profile. In this paper, the clinical experience with gemcitabine-based chemoradiation in the treatment of patients with LA-SCCHN is reviewed.

Design: We conducted a review of the literature on the clinical experience with radiotherapy combined with either single agent gemcitabine or gemcitabine/cisplatin-based polychemotherapy for the treatment of patients with LA-SCCHN. We also searched abstracts in databases of major international oncology meetings from the last 20 years. A meta-analysis was performed to calculate pooled proportions with 95% confidence intervals (CIs) for complete response rate and grade 3-4 acute mucositis rate.

Results: A total of 13 papers were fully eligible for the literature review (8 trials using gemcitabine as a single-agent and 5 trials on gemcitabine/cisplatin combinations). For schedules using a gemcitabine dose intensity (DI) below 50 mg/m^2/week the complete response rate was 86% (95% CI: 74%-93%) with grade 3-4 acute mucositis rate of 38% (95% CI: 27%-50%) and acceptable late

toxicity. In one of the studies employing such low DIs, survival data were provided showing a 3-year overall survival of 50%. Compared with DI ≥ 50 mg/m^2/week, there was no difference in the complete response rate (71%; 95% CI: 55%-83%; $p = 0.087$) but a significantly higher ($p < 0.001$) grade 3-4 acute mucositis rate of 74% (95% CI: 62%-83%), often leading to treatment interruptions (survival data provided in 8 studies; 3-year overall survival: 27-63%). Late toxicity comprising mainly dysphagia was generally underreported; while information about xerostomia and skin fibrosis was scarce.

Conclusions: This review highlights the remarkable radiosensitizing potential of gemcitabine and suggests that even very low doses (less than 50 mg/m^2/week) provide a sufficient therapeutic ratio and therefore should be further investigated. Refinements in radiation schemes, including intensity-modulated radiation therapy, in combination with low-dose gemcitabine and targeted agents, such as cetuximab, are currently being investigated.

Introduction

Concurrent chemotherapy and radiation (CCRT) is the standard treatment for locoregionally advanced (LA) squamous cell carcinoma (SCCHN) of the head and neck (oral cavity, oropharynx, hypopharynx, and larynx) [1-4]. Platinum-based CCRT, in particular, is considered standard not only for unresectable LA-SCCHN, but also for resectable yet non-surgically treated patients, and postoperative high-risk patients [5-9]. Platinum-based CCRT leads to better locoregional control, better larynx preservation and improves survival compared to radiotherapy alone [10-14]. However, the down side of this approach is the increased acute and late toxicity, which may lead to treatment-related deaths or at a later time to non-cancer related deaths [15-17].

Concerning the concurrent chemotherapy, single agent cisplatin 100 mg/m^2 on days 1, 22, and 43 is currently considered the optimal approach in carefully selected patients [12, 13]. The role of induction chemotherapy is still not fully elucidated. At this moment there is no evidence that sequential administration of induction chemotherapy followed by CCRT is superior to CCRT alone, although some signals for a benefit in oropharynx cancer patients and patients with bulky nodes are available [18, 19, 20].

Gemcitabine (2',2'-difluorodeoxycytidine; dFdC; Gemzar®, Eli Lilly and Company, Indianapolis, USA) is a fluorinated pyrimidine nucleoside analogue with antitumor activity against a wide variety of solid tumors, including head and neck cancer, and a favorable toxicity profile, when clinically used [21, 22]. In addition to that, it has synergistic activity with cisplatin and radiosensitizing properties [23-29]. Gemcitabine as a prodrug requires intracellular activation by phosphorylation to gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP) for its antitumor activity [30]. dFdCDP affects DNA synthesis by preventing the de novo biosynthesis of deoxyribonucleotide 5'-triphosphates (in particular deoxyadenosine triphosphate [dATP]) through inhibition of the enzyme ribonucleotide reductase. dFdCTP directly interferes with DNA synthesis in tumor cells through inhibition of DNA polymerization and incorporation of the fraudulent nucleotide into the growing DNA strand [31]. Finally, gemcitabine can be incorporated into RNA and can induce apoptosis [24,32].

Gemcitabine is rapidly cleared from the plasma with a half-life of only a few minutes. However, due the intracellular retention of the dFdCTP, the elimination is delayed up to 72 hours [33]. Moreover, the main metabolite of gemcitabine, difluorodeoxyuridine (dFdU) has a prolonged half-life and can be detected in the plasma for several days even after very low dosages of gemcitabine [33-35]. The radio-enhancement of gemcitabine is a complex phenomenon and seems dependent on drug exposure time and concentration [31, 36-38]. The key mechanism in the gemcitabine-induced radiosensitization has been stated to be the inhibition of ribonucleotide reductase, leading to depletion of dATP [31]. However, in addition to that, it has been hypothesized that the sustained presence of dFdU in the circulation after low-dose gemcitabine might contribute to the radio-enhancement effect of gemcitabine due to dFdU's own radiosensitizing properties [34, 35].

In the present review, the efficacy and tolerance of gemcitabine used together with radiation, as a single agent, or as a part of multi-agent-based chemoradiotherapy in combination with other cytotoxic agents, in the treatment of LA-SCCHN are summarized.

Methods

Search strategy

We searched literature from the National Library of Medicine and the Cochrane Library to identify relevant available articles (the last search was updated on May 5, 2015). We also performed a bibliographic search of abstracts in the field of interest presented at top scientific meetings (AACR, ASCO, ASTRO, ESTRO, ESMO and ECCO) within the past twenty years. The key words and subject terms were searched (i.e. head and neck cancer, squamous cell carcinoma, locally or locoregionally advanced, radiotherapy or radiation, chemotherapy, gemcitabine). The language of the papers was restricted to English. References in the studies were reviewed to identify any additional studies that were not indexed by the electronic database. Literature data concerning treatment of nasopharynx cancer were not included in this review.

Statistical methodology

Pooled proportions with 95% confidence intervals (CIs) for CR rate and grade 3-4 acute mucositis rate were calculated using a meta-analysis with random effects model.

A generalized linear mixed model with logit link was used to study the relationship between dose of gemcitabine, dose of cisplatin and occurrence of complete response (CR), mucosal and hematological toxicity (Figures 1-3). An interaction between dose of gemcitabine and cisplatin is added to account for a different effect of gemcitabine in the presence of cisplatin. Since data are clustered in trials, a random trial effect is entered in the model.

Data were visualized using bubble diagrams, where the size of the bubbles corresponds to the size of the trials. The statistical packages R 3.1.0 and SAS 9.4 were used for the analyses and graphs.

Results

A summary of 25 clinical trials using chemoradiotherapy with gemcitabine for the treatment of LA-SCCHN is provided in Table 1 [35, 39-62]. Out of the 25 trials, 13 studies published as full-text articles were eligible for further analysis. The exclusion criteria comprised: (i) publication as an abstract only, and trials or sub-studies that were (ii) investigational or (iii) had induction chemotherapy (ICT) as part of the therapeutic regimen. In addition, (iv) when gemcitabine was part of multi-agent-based chemoradiation in combination with other cytotoxic agents, cisplatin had to be part of the therapeutic regimen in order for the study to be eligible for further analysis. The details of the different chemoradiation schedules in the selected studies fully eligible for further analysis are given in Table 2 including types and schedules of radiation, details on gemcitabine and cisplatin cumulative doses, dose intensity (DI) and peak dose level and outcome. Table 3 provides an overview of the reported acute and late toxicity.

Studies reporting on chemoradiation with gemcitabine as single agent (Tables 2 and 3)

Eisbruch *et al.* were the first to report on a phase I study examining the dose-limiting toxicity (DLT) of standard radiotherapy concurrent with gemcitabine in patients with LA-SCCHN, mostly of oropharyngeal origin [39]. The preliminary results with 300 mg/m² weekly indicated that gemcitabine and radiotherapy with this regimen resulted in a high locoregional tumor control rate, but at the cost of an excessive mucosal and pharyngeal toxicity [40]. The severe (grade 3-4) mucosal reactions were the reason for a gradual de-escalation of the gemcitabine dose from 300 mg/m² to 10 mg/m² weekly. At the dose levels of 50 to 300 mg/m², a high rate of acute and in particular late toxicity was observed and one grade 5 pharyngeal toxicity reported in the 300 mg/m^2/week group. In the cohorts receiving 300 and 150 mg/m² of gemcitabine, confluent mucositis started during the third treatment week and lasted on average 7 weeks. In comparison, patients receiving 50 mg/m² developed confluent mucositis later during the treatment schedule (week 4-6 during radiation) and lasted shorter (3 weeks on average). Noteworthy most patients receiving 50 mg/m² up to 300 mg/m² required gastric feeding tubes (100% - 300 mg/m²; 92% - 150 mg/m²; 83% - 50 mg/m²) and despite trials of esophageal dilatation, the need of gastric tube feeding persisted in 50%, 17% and 33% of patients receiving 300, 150 and 50 mg/m^2/week, respectively. An important message of this study is that the researchers observed cases of late pharyngeal toxicity quite early after the treatment completion, which proved crucial for correct and timely dose de-escalation. In this particular phase I trial, no acute or late DLTs were observed at the 10 mg/m²/week dose level [39].

In a phase II study reported by Aguilar-Ponce two groups of patients with primarily tumors of the larynx and paranasal sinuses received gemcitabine either 100 mg/m^2 or 50 mg/m^2 weekly during radiation. CR rates were similar, but excessive mucosal toxicity and one treatment-related death in the higher dose group forced the researchers to lower the dose to 50 mg/m^2. Noteworthy, grade 3-4 lymphopenia occurred in 74% of patients in contrast to grade 3-4 neutropenia observed in 11% [41].

Shaharyar et al. evaluated the efficacy and toxicity of weekly administration of gemcitabine 150 mg/m² during radiation in 39 patients with LA-SCCHN. Thirty-eight percent and 28% of the primary tumors were located in the oral cavity and hypopharynx, respectively. Although the overall response rate was high (94%), acute toxicities led to treatment interruption in 41% despite vigorous symptomatic and supportive care. One treatment-related death occurred [42].

In a phase II single-institution feasibility study assessing the combination of weekly gemcitabine 100 mg/m² concomitantly with radiotherapy in patients with LA-SCCHN (in particular stage IV hypopharynx) by Specenier et al., an overall response rate of 100% was reported. In this study, grade 3-4 mucositis, pharyngitis and dermatitis occurred in 85%, 81% and 69% of patients, respectively [43]. In a later update of this study (a non-randomized comparison the same CCRT preceded by ICT), it was mentioned that none of these patients alive and without locoregional recurrence at one year were tube feeding dependent. The use of ICT made this worse (33% dependent at one year) [44].

In a prospective, randomized trial by Chauhan et al., eighty patients with LA-SCCHN (mostly of oropharyngeal origin) were randomly assigned to receive radiotherapy alone (n=40) or radiation plus gemcitabine, given at a dose of 100 mg/m²/week. There was no benefit in terms of local control, yet a significant difference in disease-free survival in favor of the combined treatment, but at the cost of more severe acute toxicity (skin, mucosa, weight loss). Importantly, in the same publication, Chauhan et al. mentioned that when they used a higher dose intensity of 200 mg/m^2/week they were confronted with an unacceptable toxicity profile (grade 3 mucositis in 100% of patients, one toxic death) [45].

A dose of 50 mg/m² of gemcitabine during radiation was used by Ali et al. in 52 LA-SCCHN patients, mostly with laryngeal cancer. They reported an 88% overall response rate (and a 67% CR rate), yet a 2-year disease-free survival of only 38% [46]. Halim et al. randomly assigned LA-SCCHN cases to CCRT either with gemcitabine or paclitaxel. They found a modest but statistically significant advantage of paclitaxel both in terms of safety and efficacy. The overall response rate in the gemcitabine-arm was 78% with 80% of patients completing the treatment program [47].

Most recently, Popovtzer together with Eisbruch and others reported on a phase I trial attempting to raise the therapeutic ratio of gemcitabine-based CCRT (intensity-modulated radiotherapy) by using a twice weekly drug regimen. They respectively explored 50, 33, 20 and 10 mg/m^2 twice weekly during the last two weeks of radiotherapy. Despite favorable data from preclinical studies suggesting that the maximally tolerated dose would be between 75-90 mg/m^2/dose when given twice weekly, the observed increase was only marginal in comparison to the once weekly schedule (2*20 mg/m^2 versus 1*10 mg/m^2) [39, 48]. Importantly, the 2-year survival rate of 41% was similar between the lower (10 or 20 mg/m^2) and higher (33 or 50 mg/m^2) dose groups, while none of the patients receiving 10 mg/m^2 experienced a DLT or required a dose-modification [48].

Studies reporting on chemoradiation with gemcitabine as part of multi-agent chemotherapy during radiotherapy (Tables 2 and 3)

In a feasibility study by Benasso et al., alternating chemoradiation with cisplatin and gemcitabine (800 mg/m^2) was responsible for an unacceptably high incidence of toxicity (mostly hematological and mucosal), leading to 3 treatment-related deaths and necessitating dose reductions of gemcitabine in 79% of patients [49].

In 2004, the same authors reported on a modification of the previous regimen in 47 patients with LA-SCCHN. The high acute toxicity rate was deemed manageable. Predominantly in terms of locoregional control, the inclusion of gemcitabine into the alternating regimen seemed to be better as compared to the results from the authors' own database on previously used alternating cisplatin-fluorouracil regimens [50].

Subsequently, in 2006, a third Italian trial further modified the alternating chemoradiation program aiming to reduce the side effects while preserving efficacy. Although both this study and the 2004 trial from Benasso et al. reported an important decrease in hematological toxicity (grade 3-4 neutropenia) compared to the first Benasso et al. study from 2001, there was no major change in non-hematological (local) toxicity. Moreover, two and one patients died during the treatment as result of neutropenic fever leading to a toxic death rate of 2% and 7% in the 2004 and 2006 trials, respectively [50, 51].

In 2008, Benasso et al. reported on a phase II trial of low-dose gemcitabine (50 mg/m²) and radiation which alternated with three courses cisplatin and 5-fluorouracil (see Table 2). As compared to their 2004 study, the frequency of severe mucositis, dermatitis and leukopenia decreased without negatively affecting the survival rates. However, the grade 3-4 mucosal toxicity tended to be higher than that of the authors' previous results with the same regimen without gemcitabine (40% versus 25%, p=0.06) and two patients died during the therapy [52]. Notably, the same dose of gemcitabine in an uninterrupted CCRT schedule was found to induce acute grade 3-4 mucositis in 75% of evaluable patients [41] (Table 3).

In order to define the maximally tolerated dose and the DLT of weekly gemcitabine and cisplatin during CCRT in patients with LA-SCCHN, Viani et al. performed a phase I study and concluded that the recommended phase II dose was 10 mg/m² and 30 mg/m² for gemcitabine and cisplatin, respectively, leading to an acceptable tolerability and compliance with 83% of patients completing the therapy without interruptions. These encouraging results may be partly due to the uniformly very good performance status (PS 0) of the patients that participated into the study [53]. Most recently, the dosing schedule corresponding to dose level 3 (20 mg/m² of gemcitabine plus 30 mg/m² of cisplatin) that Viani et al. thought to be unsuitable for further exploration in a phase II protocol, was prospectively studied by Gaur et al. in a cohort of 30 patients. Apparently, a high overall response rate was achieved (100%), but indeed at the cost of grades 3 mucositis and dermatitis in 73% and 37% of patients, respectively [54].

Comparison between very low-dose (< 50 mg/m²/week) and higher-dose (≥ 50 mg/m²/week) gemcitabine-based chemoradiation

For schedules employing very low-dose gemcitabine, a meta-analysis revealed a pooled proportion of CR rate of 86% (95% CI: 74%-93%) with grade 3-4 acute mucositis rate of 38% (95% CI: 27%-50%) and acceptable late toxicity [39, 52, 53]. In one of the studies using such low DI, survival data were provided showing a 3-year overall survival of 50% [52]. Studies number 4 and 13 have not been included in the meta-analysis because in these studies, only global response rates are given instead of a dose-specific response rate [41, 48].

For higher DIs ($\geq$ 50 mg/m^2/week), the CR rate was 71% (95% CI: 55%-83%) with a significantly greater ($p < 0.001$) severe acute mucositis rate of 74% (95% CI: 62%-83%), often leading to treatment interruptions [39, 41-43, 45-47, 49-51]. At those DIs, we did not find any correlation between the cumulative dose of gemcitabine and the severity of acute local toxicity. In terms of the CR rate, the difference between very low-dose and higher-dose gemcitabine did not reach statistical significance ($p = 0.087$). Correspondingly, based on survival data provided in 8 studies testing higher-dose gemcitabine, a 3-year overall survival ranged from 27 to 63% [41, 43, 45-47, 49-51].

Table 1. Summary of gemcitabine as single-agent or as part of multi-agent chemotherapy combined with radiation for the treatment of locally advanced SCCHN, with or without induction chemotherapy.

	Study	Primary investigator, Year	Study design	N	Anticancer Agent(s), dose	Eligibility for further analysis: Yes / No (exclusion criterion)
CCRT with GEM alone	1	Eisbruch [40], 1997	Phase I trial	8	GEM 300 mg/m^2	No (abstract)
	2	Wildfang [55], 2000	Phase II trial	18	GEM 200 mg/m^2	No (abstract)
	3	Eisbruch [39], 2001	Phase I trial	29*	GEM dose de-escalated (300 – 10 mg/m^2)	Yes
	4	Aguilar-Ponce [41], 2004	Phase II trial	27	GEM 100 or 50 mg/m^2	Yes
	5	Shaharyar [42], 2006	Phase II trial	39	GEM 150 mg/m^2	Yes
	6	Specenier [43], 2007	Phase II feasibility study	26	GEM 100 mg/m^2	Yes
	7	Chauhan [45], 2008	Prospective randomized trial	80 (40/40)	GEM 100 mg/m^2	Yes
	8	Specenier [44], 2009	Retrospective comparison	58 (31/27)	GEM 100, 50, or 10 mg/m^2 +/- platinum-based ICT	No (ICT; patients in the CCRT arm already included in the 2007 trial [43])
	9	Specenier [35], 2011	Investigational study	34 (9/8/8/9)	GEM 5, 10, 50, or 100 mg/m^2	No (Investigational study)
	10	Ali [46], 2011	Clinical investigation	52	GEM 50 mg/m^2	Yes
	11	El Deen [56], 2012	Clinical investigation	28	GEM 100 mg/m^2 + ICT with GEM and CIS	No (ICT)
	12	Halim [47], 2012 (data shown only for the GEM-arm)	Phase III	110	GEM 100 mg/m^2	Yes
	13	Popovtzer [48], 2014	Phase I trial	25	GEM dose escalated (10 – 50 mg/m^2)	Yes
	14	Ali [57], 2014 (data shown only for the GEM-arm)	Phase III	30	GEM, dose not reported in the abstract	No (abstract)
	15	Jaremtchuk [58], 2000	Phase I trial	25	GEM dose escalated to 125 mg/m^2 + Amifostine 200 mg/m^2	No (abstract)
chemoradiation with GEM and CIS	16	Benasso [49], 2001	Feasibility study	14	GEM 800 mg/m^2, CIS 20 mg/m^2 + adjuvant chemotherapy with CIS and 5-FU	Yes
	17	Benasso [50] , 2004	Phase II trial	47	GEM 300 and 800 mg/m^2, CIS 20 mg/m^2	Yes
	18	Numico [51], 2006	Clinical investigation	28	GEM 800 mg/m^2, CIS 20 mg/m^2	Yes
	19	Benasso [52], 2008	Phase II trial	47	GEM 50 mg/m^2, CIS 20 mg/m^2, 5-FU 200 mg/m^2	Yes
	20	Viani [53], 2011	Phase I trial	12	GEM dose escalated (10 – 20 mg/m^2), CIS dose escalated (20 – 30 mg/m^2)	Yes

	21	Aguilar-Ponce [59], 2013	Clinical investigation	28 (11/17)	GEM 100 mg/m^2, CIS 50 mg/m^2 +/- ICT with CIS and 5-FU	No (ICT; insufficient data reported)
	22	Gaur [54], 2014	Clinical investigation	30	GEM 20 mg/m^2, CIS 30 mg/m^2	No (abstract)
CCRT with GEM and other agents	23	Milano [60], 2004	Phase I trial	72	GEM dose escalated (50 – 300 mg/m^2), paclitaxel 100 mg/m^2, 5-FU 600 mg/m^2	No (no cisplatin in combination)
	24	Granados García [61], 2011	Clinical investigation	17	GEM 50 mg/m^2, cetuximab 400 initially, then 250 mg/m^2	No (no cisplatin in combination)
	25	Specenier [62], 2014	Clinical investigation	25	GEM 10 mg/m^2, cetuximab 400 initially, then 250 mg/m^2 +/- platinum-based ICT	No (no cisplatin in combination)

Abbreviations: CCRT, concurrent chemoradiotherapy; GEM, gemcitabine; CIS, cisplatin; ICT, induction chemotherapy; MTD, maximally tolerated dose; 5-FU, 5-fluorouracil; NR, not reported; *, one patient had medullary thyroid cancer

Table 2. Details and outcome of the chemoradiation schedules in the selected studies, including the respective sub-studies fully eligible for further analysis (see text and Table 1 for criteria of eligibility).

Sub-study	N	Overall chemoradiation schedule	GEMCITABINE			CISPLATIN			CR (%)	3-y LRC	3-y OS
			CD (mg/m^2)	DI (mg/m^2/week)	PD (mg/m^2/d)	CD (mg/m^2)	DI (mg/m^2/week)	PC (mg/m^2/d)			
3.1	8	- Standard radiotherapy, once daily, 2.0 Gy/fraction, 5 days/week; 70 Gy over 7 weeks - GEM IV 1 day/week, 4 hrs before radiation, 7 weeks concurrent with radiation 300 mg/m^2 and subsequent dose de-escalation in cohorts of at least 3 patients	2100	300	300				88	NR	NR
3.2	12		1050	150	150				80	NR	NR
3.3	6		350	50	50				67	NR	NR
3.4	3		70	10	10				67	NR	NR
4.1	15	- Standard radiotherapy, once daily, 2.0 Gy/fraction, 5 days/week; 70 Gy over 7 weeks - GEM IV 1 day/week, 1-2 h before radiation, 7 consecutive weeks at 100 mg/m^2 (n=15) and 50 mg/m^2 (n=12)	700	100†	100				61‡	NR	33
4.2	12		350	50	50						
5.0	39	- Conventional radiotherapy; to a total dose of 66-70 Gy - GEM IV 1day/week, first 6 weeks, 2h before radiation	900	129	150				23	NR	NR
6.0	26	- Conventional radiotherapy, 2.0 Gy/fraction, 5 days/week; 70 Gy over 7 weeks - GEM IV 1day/week, 2h before radiation, 7 consecutive weeks at 100 mg/m^2	700	100	100				50	NR	27
7.2	40	- Conventional radiotherapy, 2.0 Gy/fraction, 5 days/week, to a total dose of 64 Gy - GEM IV 1day/week, 1-2 h before radiation, 6 consecutive weeks at 100 mg/m^2	600	100	100				63	NR	63¶
10.0	52	- Radiotherapy, 2.0 Gy/fraction, 5 days/week; 70 Gy over 7 weeks - GEM IV 1day/week, 1 - 2 h before radiation, 7 consecutive weeks at 50 mg/m^2	350	50	50				67	NR	38#¶
12.0	110	- Radiotherapy, 2.0 Gy/fraction, 5 days/week; 65 Gy over 6.5 weeks - GEM IV 1day/week, 1 - 2 h before radiation, 6 consecutive weeks at 100 mg/m^2	600	100	100				NR	NR	45
13.1	4	- Intensity-modulated hyperfractionated radiotherapy, 1.2 Gy/fraction, twice daily, 5 days/week; to a total dose of 76.8 Gy over 6.5 weeks - GEM IV 2 days/week, during the last 11 days of radiotherapy, 5 doses in total, starting dose of GEM 10 mg/m^2, subsequent dose escalation	250	125	50				60§	NR	41#
13.2	3		165	83	33					NR	
13.3	8		100	50	20					NR	
13.4	6		50	25	10					NR	
16.0	14	- Standard radiotherapy during weeks 2, 3, 4, 6, 7 and 8; up to 60 Gy - GEM at doses of 800 mg/m^2, day 5, weeks 1, 2, 3, and 5, 6, 7, and CIS, 20 mg/m^2/d, days 1 to 5, weeks 1 and 5; at end of combined therapy, two additional courses of CIS, 20 mg/m^2/d, and 5-FU, 200 mg/m^2/d, for 5 days every 21 days	4000	500	800	240	22	20	91	70	63
17.0	47	- Standard radiotherapy once daily, 2.0 Gy/fraction, 5 days/week during weeks 2, 3, 5, 6, 8 and 9; 60 Gy over 10 weeks - GEM 800 mg/m^2, day 1, weeks 1, 4, 7, and 10, and GEM 300 mg/m^2, day 1, weeks 2, 3, 5, 6, 8, and 9; CIS 20 mg/m^2/d, days 2 to 5, weeks 1, 4, 7, and 10	5000	500	800	320	32	20	87	64	43
18.0	28	- Radiotherapy administered at 2.0 Gy/fraction, 5 days/week, on weeks 2, 3, 5, 6, 8, and 9; 60 Gy over 9 weeks	4800	533	800	240	27	20	85	64	43

		- GEM 800 mg/m^2, days 1 and 12 of every CIS course; CIS 20 mg/m^2, days 2 to 5, weeks 1, 4, and 7									
19.0	47	- Radiotherapy administered at 2.0 Gy/fraction, 5 days/week, on weeks 2, 3, 5, 6, 8, and 9; 60 Gy over 9 weeks - GEM, 50 mg/m^2, day 1, weeks 2, 3, 5, 6, 8, and 9; CIS, 20 mg/m^2/d and 5-FU 200 mg/m^2/d, days 1-5, weeks 1, 4 and 7	300	33	50	300	33	20	89	54	50
20.1	3	- Conformal radiotherapy at 2.0 Gy/fraction, 5 days/week; 70 Gy over 7 weeks - Starting dose of GEM and CIS 20 mg/m^2 and 10 mg/m^2, respectively; subsequent dose escalation of 10 mg/m^2 CIS for 3 new patients only; dose escalation of GEM with 10 mg/m^2 for each new cohort	70	10	10	140	20	20	100	NR	NR
20.2	3		70	10	10	210	30	30	67	NR	NR
20.3	6		140	20	20	210	30	30	83	NR	NR

Abbreviations: GEM, gemcitabine; IV, intravenously; CD, calculated or reported cumulative dose; DI, calculated or reported dose intensity; PD, daily peak dose of gemcitabine and cisplatin; CR, complete response in patients eligible for response evaluation; 3-y LRC, 3-year locoregional control; 3-y OS, 3-year overall survival; NR, not reported; *, one patient had medullary thyroid cancer; †, at least 80% dose intensity was achieved only in 48% of patients; ‡, there was no significant difference between the 2 arms; §, data of the whole cohort of patients; ¶, disease free survival; #, 2-year survival data

Table 3. Overview of the reported acute and late toxicities in the eligible studies and sub-studies (see text and Table 1 for criteria of eligibility) in patients evaluable for toxicity.

Sub-study	Acute grade 3 to 4 toxicity (%)				Definition of late toxicity	Late grade 1 to 2 toxicity (%)			Late grade 3 to 4 toxicity (%)		
	mucosal	pharyngeal	skin	hematological*		mucosal	pharyngeal / esophageal	other	mucosal	pharyngeal / esophageal	other
3.1	50	50	88	0	> 3 months from treatment completion	50	0	50/75§	50	75	50/13§
3.2	92	92	75	0		50	42	50/83§	50	58	42/0§
3.3	83	83	17	0		83	67	50/50§	17	33	17/17§
3.4	0	0	0	0		100	100	100/100§	0	0	0/0§
4.1	73	46	0	13/73†	NR	NR	NR	85/NR¶	NR	NR§§	NR
4.2	75	41	0	8/75†							
5.0	77	18	3	0	NR	NR	NR	NR/82§	NR	9	NR
6.0	85	81	69	8	> 3 months from treatment completion	NR	NR	74/NR§, 74/NR¶	NR	NR	9/NR§, 26/NR¶
7.2	68	NR	58	NR	NR	NR	NR	NR	NR	NR	NR
10.0	77	23	4	4	NR	NR	NR	65/NR¶	NR	NR	NR
12.0	36	NR	24	2/2/NR‡	NR	NR	NR	NR	2#	8¶¶	NR
13.1	50	75	0	0	> 1 months from treatment completion	50	25	100/75§, 100**	50	75	0/25§, 0**
13.2	67	67	67	33		33	33	100/100§, 100**	67	67	0/0§, 0**
13.3	63	100	88	0		62	75	87/75§, 100**	38	25	13/25§, 0**
13.4	50	67	33	0		100	83	67/83§, 100**	0	17	33/17§, 0**
16.0	100	NR	14	79/35/57‡	NR	NR	NR	NR	NR	NR	NR
17.0	81	NR	30	44	at 24 months from treatment completion	NR	NR	29/24¶	0	0	6/6¶
18.0	89	NR	0	46	NR	NR	NR	29/NR¶, 18##	11	NR	NR
19.0	40	NR	4	26/17/30‡	at 19 months from treatment completion	NR	NR	39/9¶	0	0	0
20.1	0	NR	0	0	NR	NR	NR	NR	NR	NR	NR
20.2	0	NR	0	33	NR	NR	NR	NR	NR	NR	NR
20.3	33	NR	17	17	NR	NR	NR	NR	NR	NR	NR

Abbreviations: *, subclassification according to the respective types of hematological abnormalities when an overall rate of hematological toxicity is not provided; †, neutropenia/lymphopenia; ‡, neutropenia/anemia/thrombocytopenia; §, skin/subcutaneous tissue; ¶, xerostomia/taste dysfunctions; #, 9 patients required artificial feeding for more than 6 months; **, laryngeal; ##, soft tissue edema; §§, symptomatic esophageal stricture (no information on toxicity grade); ¶¶, enteral or parenteral feeding necessary for more than 6 months (no further specification)

Discussion

Concurrent chemoradiotherapy with single agent cisplatin is generally accepted as the most appropriate treatment for patients with LA-SCCHN. However, long-term outcomes in the majority of cases are still far from satisfactory. Moreover, the therapy-induced morbidity due to frequent acute and late adverse events has a negative impact on the quality of life of such patients. A recent meta-analysis of three large trials in LA-SCCHN patients treated with cisplatin-based CCRT indicated that 43% of the patients suffered from severe late side effects with long-term feeding tube dependency, pharyngeal and laryngeal dysfunction occurring in 20%, 27% and 12% of patients, respectively [16]. Therefore, strategies in head and neck oncology focus not only on improvement of treatment efficacy, but also on improving its safety profile. In this review we explored the therapeutic potential of gemcitabine at both cytotoxic and radiosensitizing doses to illustrate the historical background and outline future perspectives. In total, 25 clinical studies on gemcitabine-based CCRT in LA-SCCHN were identified (14 using gemcitabine as a single agent, 11 in combination), of which 13 were eligible for further analysis.

When considering an appropriate gemcitabine-based CCRT regimen, several important factors have to be taken into account. These include: single-agent versus multi-agent chemotherapy with or without molecularly targeted agents; administration schedule; cumulative dose, dose intensity and peak dose of each drug; characteristics of radiation delivery (dose, fractionation schedule, field size); acute and late side effects along with existing possibilities for their management; short and long-term outcomes, and quality of life of the cancer survivors. The rationale for using low-dose gemcitabine schedules to enhance the effect of radiation is supported by preclinical findings demonstrating the radiosensitizing properties of gemcitabine at non-cytotoxic concentrations, and several clinical reports on gemcitabine’s efficacy in that setting at weekly doses well below those used alone or in combination with other cytotoxic agents in solid tumor treatment (10-50 mg/m^2 versus 1000-1250 mg/m^2), while higher weekly doses of gemcitabine (≥50 mg/m^2) during chemoradiation in patients with SCCHN lead to unacceptable toxicity rates [31, 39-54].

Unfortunately, gemcitabine does not elicit selective tumor radiosensitization. Despite the mild intrinsic toxicity of gemcitabine to mucous membranes, the presented literature data revealed severe acute mucositis rate of 74% (95% CI: 62%-83%) with ≥ 50 mg/m^2/week of gemcitabine, often necessitating treatment interruptions [39, 41-43, 45-47, 49-51]. In contrast, in a large sample of 342 laryngeal cancer cases assigned equally to radiotherapy with concurrent 3-weekly cisplatin 100 mg/m^2 and radiotherapy alone, the rates of grade 3-4 acute mucositis were 43% and 24% in both cohorts, respectively [8]. Moreover, there seems to be no clear correlation between the cumulative dose of gemcitabine above 300 mg/m^2 (roughly corresponding to 50 mg/m^2/week) and the severity of acute mucosal toxicity (Figure 1). Noteworthy, our meta-analysis demonstrated a statistically significant difference in severe acute mucositis rate between very low-dose (< 50 mg/m^2/week) and higher-dose (≥ 50 mg/m^2/week) gemcitabine-based chemoradiation schedules (38% versus 74%, $p < 0.001$). In addition, while acute pharyngeal and skin reactions are quite prevalent in the analyzed series, hematological toxicity is not of any importance when gemcitabine is used alone, but typically arises when it is combined with cisplatin this setting (Table 3, Figure 2). In a phase I trial setting, the radio-enhancement of gemcitabine was also explored in combination with other cytotoxic agents (paclitaxel and 5-fluorouracil). However, the therapeutic potential was again hampered by severe acute local toxicity [60]. Therefore, separation of tumor and normal tissue radiosensitizations is one of the key elements in improving the therapeutic ratio of gemcitabine-based chemoradiation.

Notwithstanding the fact that investigators in most trials on single-agent gemcitabine-based CCRT associated with high rates of severe acute mucositis deem this side effect manageable, we advocate for an increased alertness to serious late events and meticulous reporting thereof. In multivariable analysis, severe late side effects have been linked to the following independent risk factors: older age, advanced T-stage, and tumor location in the larynx or hypopharynx [16]. As mentioned earlier, late pharyngeal toxicity developing within several months after the treatment completion led Eisbruch et al. to de-escalate the dose intensity of gemcitabine [39]. Although 10 out of the 13 eligible studies presented data on late toxicities, the extent and detail of information provided differ substantially, both in terms of definition of what was considered late toxicity as well as in terms of reporting which grade was considered severe [39, 41-43, 46-48, 50-52]. Late toxicity comprising mainly mucosal and pharyngeal/esophageal toxicity was generally underreported, while information about xerostomia and skin fibrosis was scarce. From a general point of view, late radiation effects are defined as those occurring in the follow-up period greater than 90 days (Common Terminology Criteria for Adverse Events version 4.0). However, the ratios between patients evaluable for late toxicity and those evaluable for response which is usually assessed within 1-3 months after treatment discontinuation were 22/35, 17/39, 23/38 in trials published by Shaharyar et al. and Benasso et al. (2004 and 2008), respectively [42, 50, 52]. Other investigators describe neither the duration of tube feeding, nor objective evaluation of swallowing function [41, 46, 51]. Taken together with data provided by Popovtzer et al. [48] and the current lack of large randomized trials focusing on long-term survival in gemcitabine-based chemoradiation trials, it seems that the safe level may in fact lie below 50 mg/m^2/week. Schedules integrating such dose levels (corresponding to cumulative doses of gemcitabine up to 300 mg/m^2) still managed to yield a CR rate of 86% (95% CI: 74%-93%) which is not statistically different from the CR rate (71%; 95% CI: 55%-83%) achieved by higher-dose gemcitabine-based chemoradiation schedules ($p = 0.087$) [39, 41-43, 45-47, 49-53] (Figure 3). We believe that very low gemcitabine doses, even as low as 10 mg/m^2, will have radiosensitizing potential, as dFdU concentrations which showed radiosensitization in vitro could be measured for days in the plasma of patients receiving such low doses of gemcitabine [36]. Yet, within that context, it remains of interest that studies in cell lines have indicated that gemcitabine has a concentration dependent radiosensitization effect [63].

Principally, there are three methods to improve the therapeutic index of low-dose gemcitabine 1) modifications in treatment schedules, 2) novel radiotherapy techniques, and 3) combination with other anti-cancer drugs. Favorable pharmacokinetics of gemcitabine permits dosing on a once- or twice weekly basis [33]. In the study reported by Popovtzer et al. [48], 2x20mg/m^2 led to 2 DLTs in 8 patients (with grade 4 mucositis and pharyngitis), however, this regimen was given in the last two and a half weeks of hyperfractionated radiotherapy and it was the only study in which intensity-modulated radiation therapy was used. For these reasons a comparison with the other studies is rather difficult. With respect to novel radiotherapy techniques, new developments such as swallowing-sparing intensity modulated radiotherapy or intensity modulated proton radiotherapy, may improve the tolerance of CCRT [64-67]. Finally, combining gemcitabine with other active agents has become a field of growing interest. Synergistic activity with cisplatin, cetuximab or gefitinib has been described [30, 68, 69]. Using standard fractionated radiotherapy, researchers from Brazil selected weekly applications of very low-dose gemcitabine (10 mg/m^2) and moderately low doses of cisplatin (30 mg/m^2) as recommended dose for further study in phase II [53]. Disappointing experience with gemcitabine 800 mg/m^2 incorporated into platinum-based alternating chemoradiotherapy regimens made Italian investigators to reduce the dose of gemcitabine ultimately to 50 mg/m^2 during standard fractionated radiotherapy and alternated this with 3 cycles of low-dose cisplatin plus 5-fluorouracil chemotherapy [49-52].

Interestingly, these investigators also reported that the addition of gemcitabine at these low doses seemed to improve outcome. They based this on the results obtained in patients treated by the same team in two consecutive controlled trials, using the same methods of reporting. The 3-years local control rate, progression-free survival and overall survival in patients with stage IV disease treated with the very low-dose gemcitabine containing alternating regimen were 54%, 45% and 50%, respectively, vs. 40%, 28% and 35%, respectively, when they used the same regimen without gemcitabine, underscoring the potential benefit of very low-dose gemcitabine in these circumstances [52]. The feasibility of these alternating regimens (instead of CCRT) represents an attractive approach in specialized institutions which needs to be further explored [70].

To overcome the higher toxicity rate associated with combined chemotherapy regimens, molecularly targeted agents have been sought to enhance the therapeutic ratio of gemcitabine-based CCRT. Two of the most promising strategies comprise EGFR inhibitors and check-point kinase 1 inhibitors. The rationale behind the latter approach is based on the knowledge that gemcitabine activates check-point kinases which regulate the cell cycle progression. Preclinical studies employing selective knock-out of check-point kinase 1 in pancreas tumor cell lines confirmed the theoretical background [71]. With EGFR inhibitors the research has advanced much further, and interesting early clinical data have already been reported (see below) following preclinical experiments showing that EGFR inhibition, particularly with cetuximab, improves the effectiveness of gemcitabine based chemoradiation [68, 69]. It is hypothesized that the synergistic effect of gemcitabine and anti-EGFR medication results from the fact that the latter is inhibiting EGFR phosphorylation and blocking the initial survival response (EGFR activation) induced by gemcitabine and thereby promoting apoptosis. Cetuximab/radiotherapy, because of its assumed better tolerance and compliance [72, 73], often serves as an alternative for cisplatin-based CCRT in patients who might have difficulties in tolerating it, albeit that a recent literature-based meta-analysis suggested inferior efficacy [74]. As the addition of cetuximab to platinum-based CCRT only adds toxicity to platinum-based CCRT without increasing efficacy [75], other cytotoxic agents with less systemic toxicity and with a different mode of action, such as gemcitabine, in combination with cetuximab and radiation are an interesting field of research.

A recent example of that is the so-called RAGE protocol, consisting of **RA**diotherapy, **G**emcitabine and cetuximab (**E**rbitux®), given to SCCHN patients that qualified for definitive treatment with CCRT. Preliminary data were presented by Specenier et al. at the 24th American Head and Neck Society (AHNS) annual meeting in New York, 2014 [62]. They reported on 25 patients treated with weekly very low-dose gemcitabine (10 mg/m^2) and cetuximab (250 mg/m^2 after an initial loading dose of 400 mg/m^2) and intensity modulated radiotherapy (cumulative dose 69.12 Gy, with simultaneous integrated boost technique), of whom 21 received this combined approach after induction chemotherapy. The median treatment duration was 44 days and 24 of the 25 patients had a treatment duration of 47 days or less, while one patient had a treatment duration of 50 days. Patients received 97% of the planned cetuximab and gemcitabine dose, and 100% of the planned radiotherapy dose. However, adequate supportive care was necessary because 17 of the 25 patients had grade 3-4 radiodermatitis and 24 had grade 3-4 mucositis, while 10 patients experienced severe weight loss (10-20%). No conclusive data were given as yet on late toxicity and efficacy. Notably, among 17 assessable patients in a study by Granados García et al., a similarly high rate of grade 3-4 acute toxicities (88% lymphopenia, 71% mucositis, 24% rash, 6% xerostomia) was induced by 50 mg/m^2 weekly doses of gemcitabine combined with cetuximab (250 mg/m^2 after an initial loading dose of 400 mg/m^2) and concurrent standard fractionated radiotherapy [61]. Eight (47%) of the 17 patients had treatment interruptions as results of grade 3-4 toxicity (2 for hematological toxicity, 4 for mucositis, 1 for skin rash and 1 for nausea and vomiting). Toxicity was resolved within one week in most patients, but in two patients it took 3 to

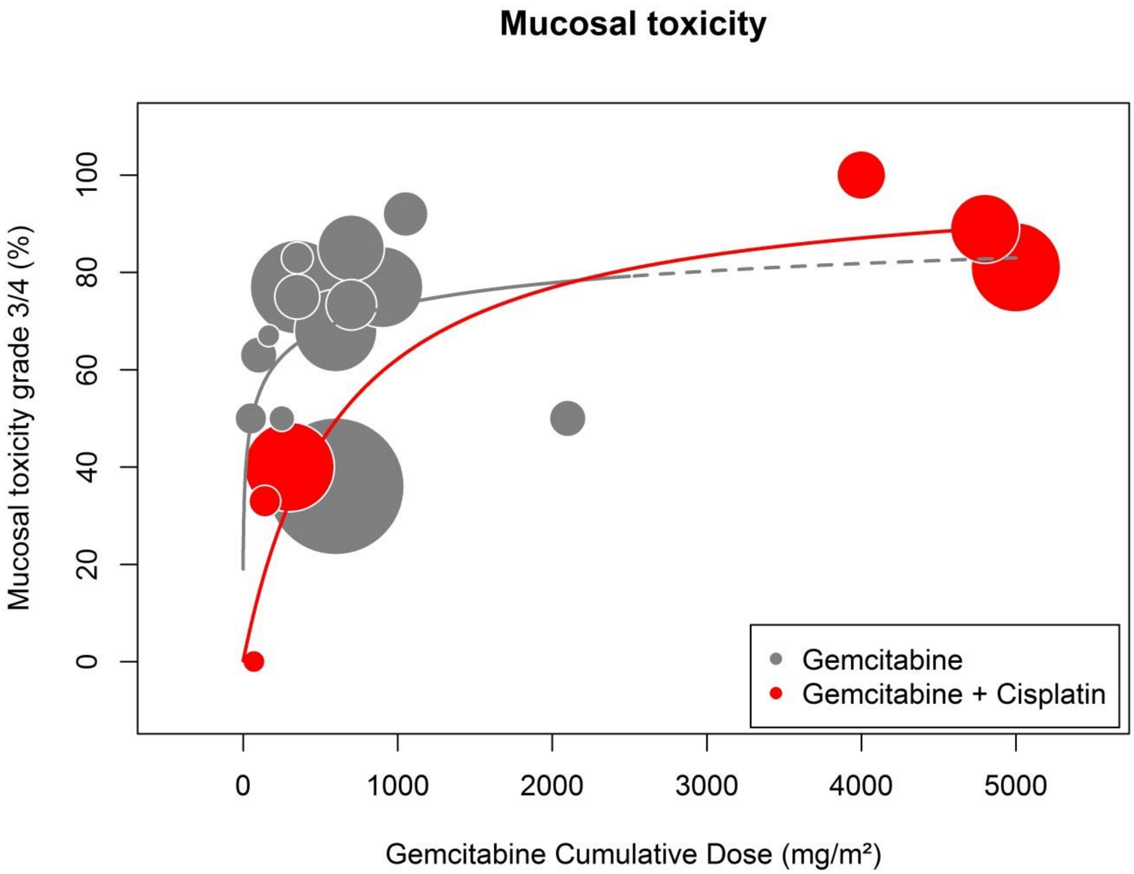
5 weeks before the severe mucositis subsided and one patient required a permanent discontinuation due to an acute abdomen. Despite the fact that toxicity was considered important, the high response rate (100%, complete 82.4%) did the investigators conclude that further studies with this triple combination were worth to consider.

Conclusions

Despite the obvious limitations given by the heterogeneity of treatment regimens and patient populations in the trials included, the presented review indicates that low-dose gemcitabine given together with radiation can produce meaningful clinical activity either as a single agent or as part of a multi-agent chemotherapy with cisplatin with or without other anti-cancer drugs. While there seems to be no difference in efficacy between very low-dose (< 50 mg/m^2/week) and higher-dose (≥ 50 mg/m^2/week) regimens, a significantly increased rate of severe acute mucositis could be observed (38% versus 74%, $p < 0.001$). The results therefore suggest that even very low doses provide sufficient therapeutic ratio and should be further investigated.

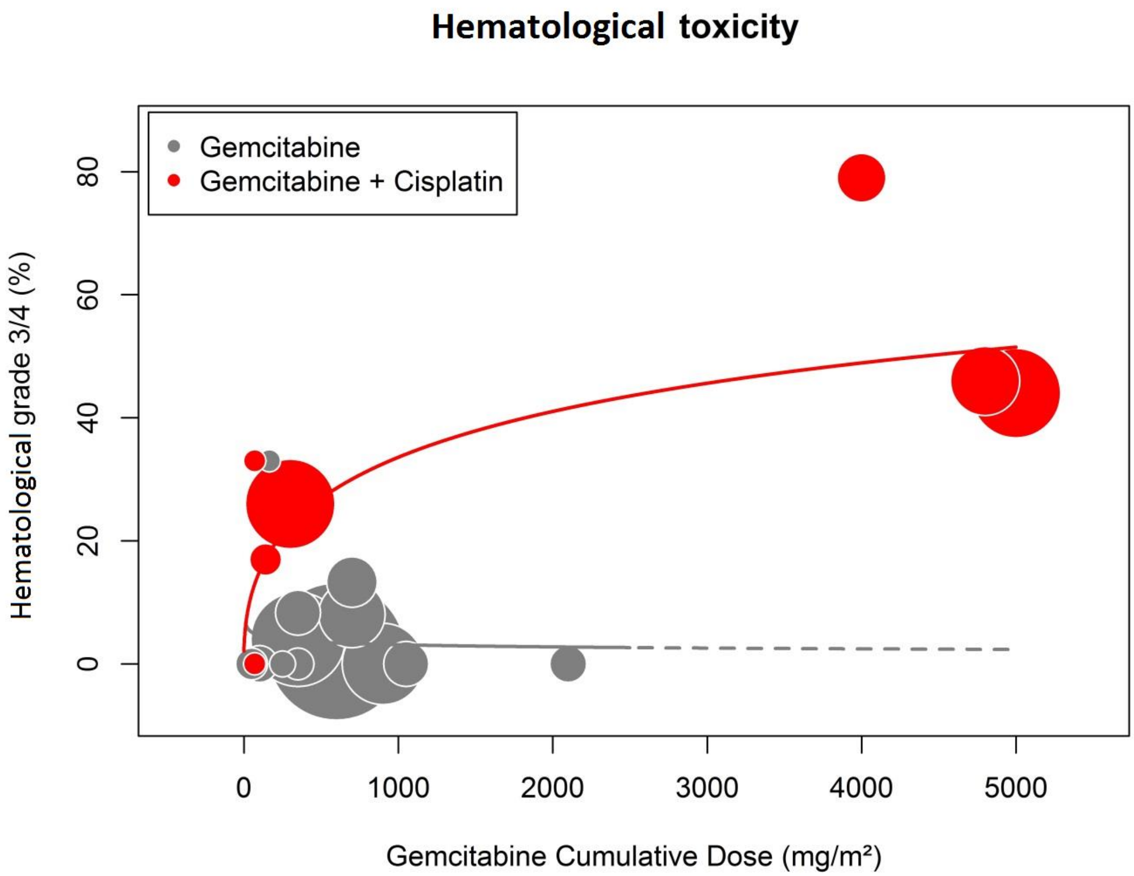
Consequently, in order to further improve the outcome in terms of both efficacy and toxicity, in particular with respect to the incidence of acute mucositis and late dysphagia, refinements in radiation schemes, the use of novel drug combinations and a better selection of patients on the basis of validated biomarkers in order to come to a more personalized treatment approach are worth considering.

Figure 1. Significant dose-related effect between cumulative dose of gemcitabine and incidence of severe acute mucositis was observed for chemoradiotherapy with gemcitabine and cisplatin (red bubbles) but not for chemoradiotherapy with gemcitabine alone (grey bubbles). However, taken together, a plateau phase above a cumulative dose of 300 mg/m^2 (roughly corresponding to 50 mg/m^2/week) suggests that no clear dose-effect correlation for mucosal toxicity exists in that segment of the curve.



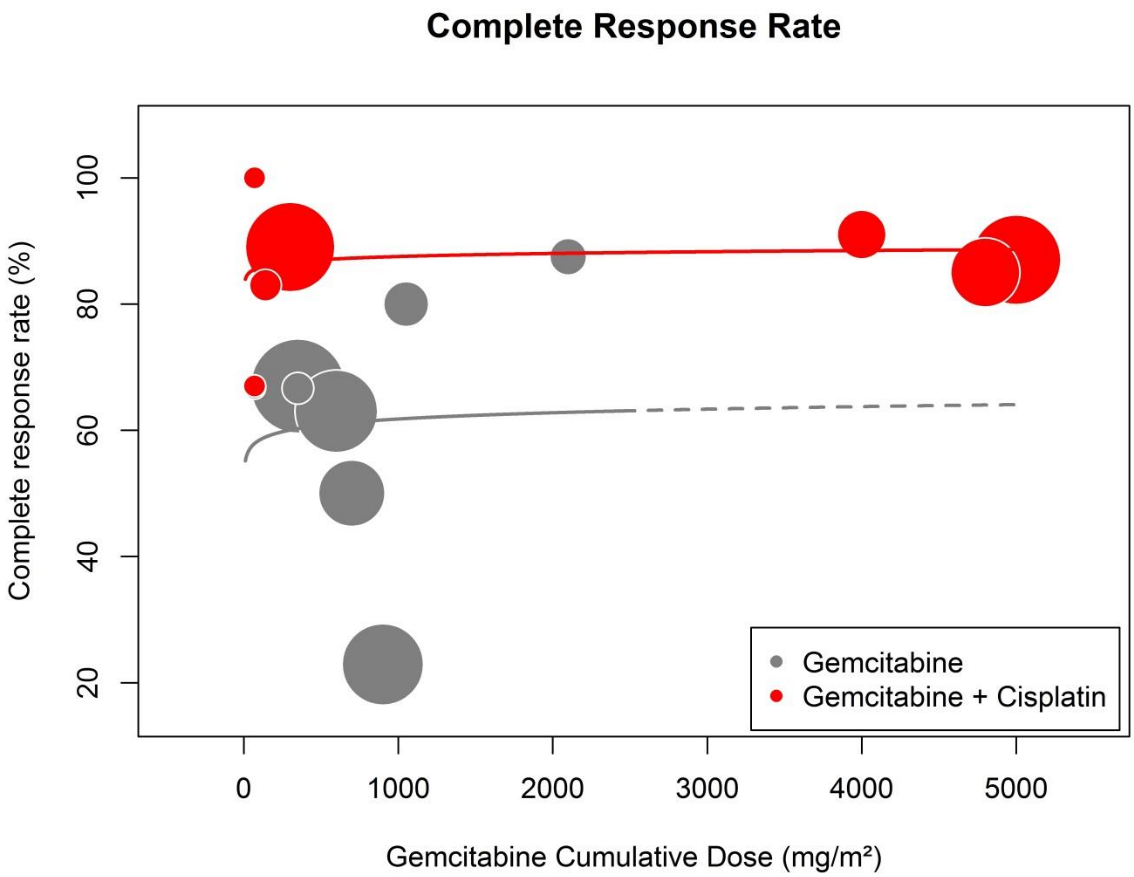
	Gemcitabine alone			Gemcitabine + Cisplatin			Comparison dose effect Gemcitabine vs Gemcitabine+Cisplatin
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P-value**	**P value**
Gemcitabine (Log10)	2.26	0.67 – 7.61	0.1871	10.37	3.20 – 33.63	0.0001	0.0773
Cisplatin				1.00	0.98 – 1.01	0.6626	

Figure 2. The incidence of severe hematological toxicity (typically neutropenia) remains low when gemcitabine is used alone (grey bubbles) but a significant dose-related effect arises when it is combined with cisplatin (red bubbles). For studies where an overall rate of hematological toxicity was not provided we used neutropenia as a surrogate marker.



	Gemcitabine alone			Gemcitabine + Cisplatin			Comparison dose effect Gemcitabine vs Gemcitabine+Cisplatin
	OR	95% CI	P value	OR	95% CI	P-value	P value
Gemcitabine (Log10)	0.66	0.07 – 6.27	0.7202	2.89	1.17 – 7.18	0.0220	0.2328
Cisplatin				1.00	0.98 – 1.01	0.6966	

Figure 3. No correlation between cumulative dose of gemcitabine and CR rate was observed. Importantly, very low-dose regimens integrating cumulative doses of gemcitabine less than 300 mg/m^2 (roughly corresponding to 50 mg/m^2/week) still managed to yield a CR rate of 86% (95% CI: 74%-93%) [39, 52, 53]. Studies number 4 [41] and 13 [48] have not been included in the analysis because in these studies, only global response rates are given instead of a dose-specific response rate.



	Gemcitabine alone			Gemcitabine + Cisplatin			Comparison dose effect Gemcitabine vs Gemcitabine+Cisplatin
	OR	95% CI	P-value	OR	95% CI	P-value	P value
Gemcitabine (Log10)	1.15	0.15 – 8.72	0.8952	1.16	0.34 – 3.99	0.8161	0.9931
Cisplatin				1.00	0.98 – 1.02	0.9220	

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