Review Article

Systemic approach to improving treatment outcome in nasopharyngeal carcinoma: Current and future directions

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Systemic therapy is an integral part of the management of non-keratinizing nasopharyngeal carcinoma (NPC). The purposes of this review are to provide the latest results and future directions of clinical and translational research for this disease, and to illustrate how some of these new therapies have improved the treatment outcome for patients with NPC. Particular attention will be paid to the clinical application of chemotherapy in the adjunctive treatment of locoregionally advanced NPC, novel targeted drugs, Epstein–Barr virus-targeted vaccine therapies, and the use of plasma Epstein–Barr virus DNA as a biomarker for selecting patients for adjunctive therapies. (*Cancer Sci* 2008; 99: 1311–1318)

Non-keratinizing nasopharyngeal carcinoma (NPC) is a unique disease in terms of its geographic distribution, biological association with the Epstein–Barr virus (EBV), and its sensitivity to chemotherapy and radiotherapy (RT). Endemic to China and the South-east Asian region, this cancer reaches a peak incidence rate of around 20 per 100 000 person-years in Hong Kong,[1] where there has been a substantial improvement in treatment outcome for NPC from 1996 to 2000 as reported in a population-based analysis by the Hong Kong NPC Study Group.[2] However, this analysis also highlighted the fact that distant recurrence is the most common cause of treatment failure following RT with a reported 5-year rate of 19% for all disease stages, and 25% for the stage III-IVB subgroup.[2,3] This trend is likely to bring about an increasing burden of metastatic cases that is largely incurable in the majority of patients. The immediate priorities lie in the identification of effective adjunctive therapies for controlling micrometastases following RT, and prolonging disease remission in patients with recurrent or metastatic NPC. The present review focuses on the recent advances in the clinical development of systemic strategies that may fulfill these priorities. These include the novel applications of cytotoxic chemotherapy, translational studies of molecular targeted agents, and vaccine therapy against EBV-associated antigens. Table 1 outlines some of the contemporary challenges of treating NPC, and the potential roles that systemic strategies may play in optimizing therapeutic outcome.

Advances in the clinical application of cytotoxic chemotherapy

Use of adjunctive chemotherapy in the curative setting. Three meta-analyses published to date have concluded unanimously that chemotherapy confers a survival advantage to conventional fractionated RT in patients with locoregionally advanced NPC.[4–6] Involving over 10 randomized trials of over 2500 patients with predominantly American Joint Committee of Cancer (AJCC)[7] stage III-IVb (T2b-4, or N3-4) NPC, two meta-analyses reported an 18% reduction in the risk of death and an absolute survival benefit of 4–6% at 5 years with the use of adjunctive chemotherapy.[4,6] Interestingly, this survival benefit was associated with a reduction of 26% in the risk of distant failure,[4,6] an observation that lends support to the hypothesis that adjunctive chemotherapy exerts its greatest clinical impact by controlling micrometastases.[4,6] In these meta-analyses,[4,6] the benefit observed with adjunctive chemotherapy was most pronounced with concurrent chemoradiation, which reportedly reduces the risk of death in stage III-IVb NPC by 40–52%. Phase III studies published from Hong Kong and Singapore subsequently confirmed this observation and found a similar magnitude of benefit in favor of concurrent chemoradiation over RT alone.[8,9] As outlined in Table 2, this benefit was evident irrespective of the type or schedule of concurrent chemotherapy used in these studies. These included high-dose cisplatin,[8,10,11] or weekly low-dose cisplatin,[12] and non-platinum agents such as tegafur-uracil (UFT).[11] Interestingly, a recent study asked the question of whether the cisplatin component in the concurrent and adjuvant therapy described in the US Intergroup study[10] can be substituted with carboplatin.[13] The investigators found no difference in overall or disease-free survival at a relatively short median follow-up of 26.3 months,[13] except that carboplatin was better tolerated and resulted in fewer mucosal and renal toxicities. However, the sample size of that study (eligible patients of 206) seemed conservative if it was intended to be a 'non-inferiority' study, especially when one compares it with the sample size of other 'superiority' studies summarized in Table 2. Only 59 and 42% of patients treated with cisplatin could complete the concurrent and adjuvant therapies, respectively, compared with over 70% of patients who received carboplatin.[13] In contrast, an exploratory analysis from a phase II study found that substitution of cisplatin with carboplatin in concurrent chemoradiation adversely affected clinical outcome.[14] Until more definitive studies are available, carboplatin should not routinely replace cisplatin in clinical practice unless a patient cannot tolerate cisplatin. In light of the compelling evidence in favor of chemoradiation for the treatment of stage III-IVb NPC, this approach is now the standard of care in Hong Kong and some parts of the world. It

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Table 1. Potential systemic strategies for improving the treatment outcome for nasopharyngeal carcinoma (NPC)

Challenges	Potential systemic strategies
Distant failure after radiotherapy in stage III-IVb NPC	– Early identification of patients at risk of distant failure using plasma Epstein–Barr virus DNA – Eradicate micrometastases with adjunctive chemotherapy, or targeted agents
Optimal schedule of adjunctive therapy with radiotherapy unclear	– Evaluate neoadjuvant chemotherapy before chemoradiation – Adjuvant chemotherapy
Improve tolerability of adjunctive therapy with radiotherapy	– Adopt modern cytotoxic agents in adjuvant therapy (e.g. gemcitabine, taxanes) – Replace cisplatin with other radiosensitizers (e.g. oral 5-fluorouracil, bevacizumab, cetuximab) – Improve supportive therapy during radiotherapy – Better radiotherapy techniques
Platinum resistance in a palliative setting	– Using multiple drugs with few cross-resistance – Use other platinum (e.g. oxaliplatin) – New non-platinum agents (e.g. gemcitabine, capecitabine, taxanes) – Overcoming platinum resistance with targeted agents
Relatively short duration of disease control from palliative chemotherapy	– Maintenance therapy following remission with platinum-based chemotherapy

Table 2. Summary of key phase III studies comparing chemoradiation and radiotherapy (RT) alone and impact on treatment outcome

First author	Year	*n*	Treatment arms	Result (all stages)			
				Survival time	CRT (%)	RT (%)	*P*-value
Al-Sarraf[10]	1998	147	RT	5 years OS	67	37	0.001
			RT + C → adj C-FU	5 years PFS	58	29	0.001
Chan[12]	2002 (2005)	350	RT	5 years OS	72	59	0.048
			RT + C (weekly)	5 years PFS	62	52	0.076
			HR: 0.71 (95% CI = 0.5–1.0) for all, HR = 0.51 (95% CI = 0.3–0.88) for T3/4				
Lin[96]	2003	284	RT	5 years OS	72.3	54.2	0.002
			RT + C-FU	5 years PFS	71.6	53.0	0.001
Kwong[11]	2004	219	RT	3 years OS	86.5	76.8	0.06
			RT + UFT	3 years FFS	69.3	57.8	0.14
			RT + UFT → adj CFVBM				
			RT → adj CFVBM				
			HR: 0.41 (95% CI, 0.21–0.78; $P = 0.007$)†				
Wee[8]	2005†	221	RT	3 years OS	80	65	0.0061
			RT + C → adj C-FU	3 years DFS	72	53	0.0093
			HR: 0.51 (95% CI, 0.31–0.81; $P = 0.0061$)				
Lee[15]	2005†	348	RT	3 years OS	78	78	0.97
			RT + C → adj C-FU	3 years FFS	72	62	0.027
			HR: not significant at 2 years				
Zhang[97]	2005	115	RT	2 years OS	100	77	0.01
			RT + oxaliplatin	2 years RFS	96	83	0.02
Lee[9]	2006	189	RT	3 years FFS	94 (aRT arm)	70	0.008
			RT + C → adj C-FU				
			aRT				
			aRT + C → adj C-FU				
			HR = 0.52 (0.28–0.97)				

adj, adjuvant; aRT, accelerated RT; C, concurrent cisplatin at 3-weekly schedule (unless specified); C-FU, cisplatin and 5-fluorouracil; CFVBM, cisplatin–fluorouracil–vincristine–bleomycin–methotrexate; CI, confidence interval; CRT, concurrent chemoradiation; DFS, disease-free survival; FFS, failure-free survival; HR, hazard ratio for death after concurrent chemoradiation over radiotherapy alone; OS, overall survival; RFS, relapse-free survival.
†Refers to a subgroup analysis.

should be emphasized that concurrent chemotherapy does exacerbate the acute and late toxicities of RT, including radiation mucositis, ototoxicity, and soft tissue damage.[15,16] Therefore, clinicians should minimize treatment-related morbidities by using better RT techniques (e.g. intensity-modulated radiotherapy [IMRT]), and streamlining patient selection for chemoradiation using more accurate methods of staging.

It is recognized that the risk of distant failure experienced by some patients with stage IIb NPC approaches that of stage III disease following RT.[17,18] However, the role of adjunctive chemotherapy for the stage IIb subgroup has not been defined as a primary endpoint by a phase III study, with some studies advocating the use of concurrent chemoradiation,[19,20] or neoadjuvant chemotherapy.[21] In a small retrospective study from Taiwan, the treatment outcome of 44 patients with stage I-II NPC was examined, of whom one was treated with RT alone and 30 with chemoradiation. A non-significant trend toward a better 3-year disease-free survival was seen favoring chemoradiation over RT alone in the stage II subgroup.[20] In our phase III study of 350 patients with NPC (of whom 101 had stage II

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Table 3. Phase III studies comparing radiotherapy with or without neoadjuvant chemotherapy

First author	*n*	Patients	Median follow up	Arms	Result (all stage)			
					Survival time	RT (%)	CRT (%)	*P*-value
Roussy[29]	339	All N2-3	49 months	RT	3 years OS	NR	NR	NS
				BEP → RT	3 years DFS	~35[†]	~55	0.01
Chua[30]	334	All N2-3	30 months	RT	5 years OS	42	48	NS
				EC → RT	5 years RFS	71	78	NS
Ma[31]	456	Some N0	NA	RT	5 years OS:	56	63	NS
				CBF → RT	5 years FFS	49	59	0.05

[†]Values obtained from a survival curve. Actual figures not provided. All are intention-to-treated data.
BEP, bleomycin, epirubicin, cisplatin; CBF, cisplatin–bleomycin–5-fluorouracil; CRT, concurrent chemoradiation; DFS, disease-free survival; EC, cisplatin, epirubicin; FFS, failure-free survival; OS, overall survival; RFS, relapse-free survival; RT, radiotherapy.

disease), a planned subgroup analysis found that the survival benefit associated with chemoradiation achieved statistical significance only in patients with T3-4 NPC (hazard ratio [HR] = 0.53, 95% CI, $P = 0.012$), but not those with T1-2 disease.[12] Nevertheless, the difference in overall and progression-free survival did reach borderline significance for the entire cohort after adjusting for T-stage, age, and overall stage. It is difficult to rule out a small survival benefit of treating stage IIb NPC with chemoradiation based on the existing evidence; therefore, until more definitive results are available, chemoradiation may be a reasonable option for selected patients with bulky stage IIb NPC who are otherwise medically fit. As discussed later, future studies should be focused on using biomarkers in selecting patients who are at risk of distant failure after RT for further adjunctive treatment.

Although the use of three cycles of adjuvant cisplatin and 5-fluorouracil (5FU) after RT, as described in the US Intergroup study,[10] is popular in some Asian and North American centers,[8,10,15] there is no direct evidence from phase III studies or meta-analyses that support its use.[11,22,23] Compliance to treatment is another problem as some phase III studies reported that up to 15% of patient did not receive any planned adjuvant chemotherapy,[8,15] because of toxicity and patient refusal.[8] In an attempt to improve tolerability, some investigators have evaluated the use of neoadjuvant (or 'induction') chemotherapy, which may theoretically control micrometastases, with the added benefit of facilitating RT planning through a 'down-staging' effect on some locally advanced tumors (e.g. T4 tumors with bulky extension to the brain or optic chiasm). Indeed, phase II studies of neoadjuvant therapy using multiagent regimens have reported some impressive response rates of up to 80%,[24–26] and retrospective studies have suggested that such an approach might improve clinical outcome over RT alone.[27,28] To date, only three adequately powered phase III studies[29–31] have exclusively compared RT with or without neoadjuvant chemotherapy in patients with non-keratinizing NPC (Table 3). The French and Asian-Pacific study included only patients with N2-3 NPC,[29,30] whereas the Guangzhou-led study included some earlier stage patients (T1-2, N0-2).[31] The French study[29] found a statistically significant improvement in disease-free survival favoring the neoadjuvant arm, but the relatively high incidence of treatment-related mortalities (14 deaths) probably contributed to the lack of difference in overall survival.[29] The Asian-Pacific group reported no difference in overall survival between the treatment arms, except that the neoadjuvant arm was associated with a better relapse-free and overall survival in a subgroup analysis of 69 patients with advanced nodal size (>6 cm).[30] Poor compliance to neoadjuvant chemotherapy and a relatively short study follow up (30 months) might have undermined the magnitude of benefit observed in the Asian-Pacific study.[30] This study's result was recently updated in a pooled analysis with a negative trial led by the Guangzhou group.[31] At a median follow up of 67 months, this analysis found that neoadjuvant chemotherapy was associated with an absolute improvement in disease-specific survival of 5.4%, and a reduction in the 5-year rates of local and distant recurrence of 18.3 and 13.3%, respectively.[32] Building on this premise, recent studies addressed the question of whether neoadjuvant chemotherapy using modern cytotoxic agents can add to the benefits of concurrent chemoradiation. At the Prince of Wales Hospital, we compared the feasibility of treating 65 patients with stage III-IV NPC with concurrent cisplatin and radiotherapy, with or without two cycles of neoadjuvant cisplatin and docetaxel in a randomized phase II study.[33] Intriguingly, early analysis at a median follow up of 2.74 years suggested that the neoadjuvant arm was associated with a significant reduction in the risk of death (HR = 0.17; 95% CI = 0.037–0.82; $P = 0.013$) and a 17% absolute improvement in 2-year overall survival. Although the study was not powered to detect a survival difference between the two arms, this encouraging result warrants confirmation in a larger phase III study. This is especially relevant as induction treatment with a docetaxel-based regimen has been shown to improve survival in a recently published phase III study in patients with non-NPC squamous cell carcinoma.[34]

The evidence as discussed above calls for an expanding role of chemotherapy in the curative treatment of advanced NPC. The most pertinent issues confronting investigators at this point in time are defining the optimal sequencing of adjunctive chemotherapy with chemoradiation, and improving our existing method of selecting patients according to disease stage alone who might benefit from more aggressive adjunctive therapy. To address these issues, the Hong Kong NPC Study Group is conducting two multicenter phase III trials involving six large oncology centers in Hong Kong. The 'NPC-0501' study randomizes 798 patients with stage III-IV NPC to one out of six treatment arms, which compare two different schedules of chemotherapy (neoadjuvant vs adjuvant chemotherapy in addition to chemoradiation), two different regimens (adjunctive cisplatin-5FU vs cisplatin-capecitabine), and two types of RT fractionation (conventional vs accelerated RT). This study is expected to accrue data over a period of around 3 years. The other phase III study is the 'NPC-0502' study, the design of which is based on the substantial body of evidence on the prognostic power of plasma EBV DNA in predicting disease recurrence following chemoradiation, which is discussed in the next section.

Using the technique of quantitative real-time polymerase chain reaction in measuring circulating DNA,[35] investigators at our university were able to demonstrate prospectively that patients with an elevated plasma EBV DNA level at 6 weeks after completing chemoradiation were 12 times more likely to experience disease recurrence than those without.[36] This finding

Table 4. Selected phase II trials of chemotherapy in first-line treatment of metastatic or recurrent nasopharyngeal carcinoma (NPC)

First author	Year	Sample size	Regimen	Overall response (%)	Time to progression (months)	Median survival (months)
Boussen[43]	1991	49	Cisplatin–bleomycin–5FU	78	–	NR
Au[98]	1994	24	Cisplatin–5–fluorouracil	66	(Median) 8	11
Siu[42]	1998	90	CAPABLE	80	–	14
Yeo[53]	1998	27	Carboplatin-paclitaxel	59	(Mean) 6	12
Taamma[44]	1999	49	Cisplatin–5FU–bleomycin–epirubicin	78 (M1) 91 (LA)	–	–
Ngan[99]	2002	44	Cisplatin–gemcitabine	73	(Median) 10.6	15
Chua[100]	2005	19	Cisplatin–docetaxel	56	(Median) 5.6	12.4
Leong[45]	2005	32	Carboplatin–paclitaxel–gemcitabine	78	(Median) 8.1	18.6
Chan[54]	2007	23§	GEMOX‡	52	–	NR

‡Biweekly oxaliplatin and infusional gemcitabine.
§Interim report only.
5FU, 5-fluorouracil; CAPABLE, cyclophosphamide, doxorubicin, cisplatin, methotrexate, and bleomycin; GEMOX, oxaliplatin and infusional gemcitabine; LA, locally advanced NPC; NR, not reached, M1, metastatic NPC.

was confirmed in a subsequently published study from Taiwan.[37] Furthermore, plasma EBV DNA may play a complementary role in the staging of NPC as our colleagues were able to show that it might be a better prognostic discriminator of patients with stage IIb NPC than the AJCC staging criteria alone.[38] Based on this background information, the NPC-0502 study was designed to address the question of whether patients with a detectable level of plasma EBV DNA at 6 weeks following chemoradiation should be offered adjuvant chemotherapy. This study is expected to proceed over 4 years and around 1500 patients who have completed RT will be screened and tested for plasma EBV DNA. Only those patients with a detectable level of plasma EBV DNA will be randomized to undergo observation alone or six cycles of adjuvant cisplatin and gemcitabine.

Use of chemotherapy in the palliative setting. The median survival of metastatic NPC varies considerably depending on the location and number of metastases and the metastasis-free interval from the time of initial diagnosis.[3,39] Although chemotherapy has never been compared with supportive care alone in metastatic NPC, platinum-based chemotherapy is a popular choice for this indication because of its association with excellent response rates and occasional reports of prolonged remissions[39] (Table 4). However, even among well-selected groups of treatment-naïve patients who received platinum-based chemotherapy in phase II trials as summarized in Table 4, the reported median survival is at best 12–18 months with a time to disease progression of 5–10 months. Platinum resistance is the main hurdle and generations of investigators have tried to overcome it with strategies such as combining drugs with minimal cross resistance, intensifying drug dosages,[40] and using other platinum with known preclinical activity against cisplatin-resistant cancer cells, such as oxaliplatin.[41] A more recent approach is to combine targeted agents with platinum, as will be discussed in the present review.

As outlined in Table 4, successive phase II studies of chemotherapy combinations in metastatic and recurrent NPC seem to show that regimens containing a higher number of agents are associated with higher response rates, longer time to progression, and also serious toxicities.[42–45] Nevertheless, one should interpret these data with caution for several reasons. Patient selection is a relevant factor because variability in survival for metastatic NPC has been well documented in large cohort studies. The patient's age and performance status, the number and types of metastases, metastasis-free interval from diagnosis, and the aggressiveness of salvage treatments (e.g. multimodal treatment of metastases such as RT or locally ablative therapies) are all important factors influencing survival.[3,39] To illustrate this point, the patients described in one of the studies that reported a large number of prolonged remissions were relatively young and fit (median age 28 years) with few sites of metastases.[46] Another reason that calls for caution when interpreting the phase II studies in Table 4 concerns the assessment of treatment response in patients with isolated locoregional recurrence. Radiologically, locally recurrent tumors are often irregularly shaped with poorly defined contours. Furthermore, radiological differentiation between recurrent tumors from post-RT changes can be difficult in some cases without a biopsy, which may not be feasible especially for deep-seated recurrence (e.g. base of skull). All of these factors complicate the process of measuring tumor response to chemotherapy in clinical trials.[47] The criteria of response assessment can also influence the reported response rate, as our colleagues have shown in a retrospective series that bidimensional response criteria (the WHO criteria) was a better indicator of change in tumor size than the popularly used unidimensional RECIST criteria for local NPC tumors.[47] Therefore, the controversy of whether multidrug regimens (e.g. triplets) should be favored over platinum-based doublets in the palliative treatment of NPC can only be clarified in a well-powered phase III trial. The study design should include the following considerations: stratification of patients according to their disease status (metastatic group vs non-metastatic, locally recurrent group), planned subgroup analyses based on known prognostic factors (e.g. the number and type of metastases), and the use of magnetic resonance imaging-defined bidimensional criteria in assessing response for local tumors. The application of plasma EBV DNA as a tool for assessing response in clinical trials should also be validated prospectively, given the promising results reported in the palliative setting.[48]

For patients with recurrent or metastatic NPC who are refractory to platinum-based chemotherapy, there is not a single chemotherapy regimen that is universally regarded as the standard of care. Phase II trials of second-line monotherapy or combinations have reported variable response rates of 14–48%,[49–52] but these results need to be interpreted with caution because of the relatively small sample size of these studies ($n = 17–39$). The list of cytotoxic agents that are active in this setting is steadily expanding and include agents such as capecitabine,[49] irinotecan,[50] vinorelbine,[51] and gemcitabine.[52] However, there is still room for improvement in terms of advancing treatment outcome, as the reported time to progression of 5 months,[49,52] and median survival of 7–11 months remain poor in these studies,[49–52] therefore newer agents are needed.

Although the prevailing opinion favors the use of platinum as the 'backbone' for all first-line regimens for NPC, there are no

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randomized data on which platinum is the agent of choice in the palliative setting. A popular choice is cisplatin because it has been well tested in phase II trials and has been associated with prolonged remissions.[46] Carboplatin is often seen as an alternative to cisplatin in patients who cannot tolerate cisplatin; however, it should be stressed that carboplatin does have proven activity in the palliation of NPC especially when it is dosed adequately,[53] and used in drug combinations.[45] Oxaliplatin has been investigated as another 'safer' alternative to cisplatin because of its lack of association with significant renal toxicity and ototoxicity. Our group found that the GEMOX regimen (oxaliplatin and infusional gemcitabine in a 2-weekly schedule) holds promise in the palliative treatment of NPC in a phase II study,[54] and oxaliplatin is currently under evaluation in combination with capecitabine by other groups.

Targeted therapy

The current model of NPC pathogenesis describes a stepwise progression from normal epithelium to a state of preinvasive dysplasia that culminates as invasive carcinoma. This transformation is thought to involve a sequence of molecular events, which include the loss of heterogeneity at specific chromosomal regions with resultant genetic mutations, EBV latent infection, epigenetic silencing of tumor-suppressor genes, and activation of certain signaling kinases.[55,56] These events serve critical functions in promoting and maintaining the malignant phenotype of NPC, and elucidation of these events holds the key to finding exploitable targets for therapeutic intervention. A detailed discussion on the molecular biology of NPC is beyond the scope of this review, and readers may refer to some excellent reviews on this topic.[55,56] In brief, genome-wide microarray studies of NPC tissues have described a high incidence of aberrant expression of genes controlling a range of important cellular processes such as the cell cycle, apoptosis, cell migration and adhesion, growth, and differentiation.[57] High levels of amplification of oncogenes such as *MYCL1*, *N-RAS*, *RAF1*, and epidermal growth factor receptor (*EGFR*) can be found in NPC tissues and cell lines.[58] EBV latent infection and epigenetic silencing of EBV immunodominant genes may enable NPC cells to evade the host's immune surveillance, whereas the production of oncogenic EBV proteins (e.g. latent membrane protein [LMP]-1) contributes to NPC carcinogenesis.[56,59] In this section, we will review how the knowledge of these molecular events may be translated clinically in the treatment of NPC.

Inhibition of signaling protein kinases. The knowledge that kinase-mediated cell signaling is commonly deregulated in epithelial cancers, and that such kinases can be pharmacologically inhibited has led to the popular development of protein kinase inhibitors in oncology. The rationale of targeting the EGFR-mediated signaling in NPC is based on both preclinical and clinical groundwork. For instance, *EGFR* gene amplifications can be found in 40% of NPC tissues,[58] and EGFR overexpression is associated with poor prognosis following chemoradiation in patients with advanced NPC.[60] Inhibition of EGFR signaling with the monoclonal antibody cetuximab,[61,62] or the tyrosine kinase inhibitor gefitinib,[63] has been shown to retard cell growth and induce apoptosis in NPC cells. Based on our work on the additive effect of combining cetuximab with platinum in NPC cell lines,[62] cetuximab and carboplatin were evaluated in combination in 60 patients with metastatic NPC who had failed previous platinum-based regimens.[64] The combination was well tolerated and the overall response rate was 11.7%, with a disease stabilization rate of 48.3%. Given the promising result of combining cetuximab and RT in non-NPC squamous cell carcinoma of the head and neck,[65] we are currently evaluating the feasibility of combining cetuximab with low-dose cisplatin and IMRT in a phase II trial of advanced NPC. Gefitinib has also been evaluated as a monotherapy (500 mg daily) in chemotherapy-refractory patients in a phase II study. The study was terminated at interim analysis owing to a lack of objective response,[66] except for three patients with disease stabilization lasting 3–8 months. This lack of response to gefitinib may be explained by the fact that activating EGFR kinase mutations have not been described in NPC yet, unlike other cancers such as adenocarcinoma of the lung.[67,68] Further a field, other protein kinases such as c-MET[69,70] and STAT-3[71] are also being investigated as potential targets for NPC. For instance, c-MET is a membrane-associated tyrosine kinase that is located upstream of several important oncogenic pathways (e.g. Ras–Raf–MAPK and β-catenin–Wnt), and is closely linked with cancer metastasis.[72] In NPC, c-MET protein overexpression is relatively common and is associated with poor prognosis in late-stage disease.[73] Investigators at our department have recently reported that c-MET activation by its ligand hepatocyte growth factor can promote cell growth and invasiveness in NPC cell lines, and both of these processes can be abrogated via inhibition of c-MET signaling.[69,70] Future clinical trials of protein kinase inhibitors should explore the role of these agents as adjuvant therapy following chemoradiation, or as maintenance following palliative chemotherapy given their preclinical effects on cell metastasis.

Hypoxia and angiogenesis. Tumor hypoxia is associated with resistance to RT and chemotherapy, and hypoxia-inducible factor (HIF)-1α is a key hypoxia-inducible transcriptional factor that upon activation, leads to the upregulation of several important hypoxia-responsive genes that regulate apoptosis, glucose metabolism (e.g. carbonic anhydrase [CA]-9), and angiogenesis (e.g. vascular endothelial growth factor [VEGF] receptor [VEGFR] and its ligands).[74] This observation has been similarly described in NPC cell lines where the expression of genes encoding HIF-1α, CA-9, VEGF, and other signaling proteins in NPC were upregulated upon exposure to hypoxia.[61] In NPC tissues, overexpression of HIF-1α, CA-9, and VEGF was found in over 50% of cases and coexpression of HIF-1α, CA-9, and VEGF was associated with poorer survival following RT in advanced NPC.[75] Therefore, inhibition of HIF-1α or its downstream targets maybe a rational strategy against NPC. Sorafenib is a multitargeted kinase inhibitor against VEGFR (VEGFR-2, VEGFR-3), platelet-derived growth factor receptor, Raf kinase, and others. This drug was evaluated in a phase II study of 27 patients with head and neck cancer, out of whom six had undifferentiated NPC. Although the sample size is too small to be conclusive, the median time to progression of 3.2 months and overall survival of 7.7 months as reported in the NPC subgroup seems relatively modest when one compares it with a historical time to progression of 4–5 months and overall survival of 7.6–10 months from phase II studies of second-line chemotherapy.[49,50,76,77] This study was terminated after the first stage of accrual because only one partial response was seen, and one patient died of nasopharyngeal bleeding, which was attributed to the underlying cancer. Other kinase inhibitors against VEGFR are currently being evaluated at ours and other centers. A monoclonal antibody against VEGF, bevacizumab, is being evaluated concurrently with IMRT in the treatment of advanced NPC in a Radiation Therapy Oncology Group-sponsored multicenter study. Given the known association of such agents with rare occurrences of serious hemorrhages in other cancers,[78] caution should be exercised when selecting patients with NPC for clinical trials of VEGF or VEGFR inhibitors, especially in those with primary tumors that invade major blood vessels, or those with premorbid symptoms of bleeding such as epistaxis.

DNA methylation and histone acetylation. One of the ways to encourage the host's immunological attack against EBV antigens in an immunocompetent host is to facilitate expression of

the immunodominant EBV nuclear and lytic antigens in NPC tumors.[79] CpG methylation of the promoters of EBV nuclear and lytic antigens has been implicated in the epigenetic silencing of these viral genes in NPC.[80] Investigators at our university also reported that silencing of host-derived tumor-suppressor genes is commonly mediated via similar epigenetic mechanisms in NPC.[81] In a proof-of-concept study, we and our collaborators were able to demonstrate for the first time in humans that the demethylating agent azacitadine can induce expression of silenced EBV genes in NPC tissues.[79] Histone acetylation is another important epigenetic mechanism of regulating gene expression, and some researchers have postulated that reversal of both promoter methylation and histone deacetylation may lead to a greater degree of gene transcription than the reversal of one mechanism alone.[82] Thus, our center and our collaborators are currently conducting a phase I study combining azacitidine and a histone deacetylase inhibitor in NPC.

Immunotherapy

Epstein–Barr virus is present in virtually all poorly and undifferentiated NPC and the viral antigens expressed by the tumor provide potential targets for immunotherapy.[83] Adoptive transfer of cytotoxic T cells (CTL) specific for EBV antigens has proved highly successful as prophylaxis and treatment for EBV-associated lymphoproliferative disease (PTLD) in bone marrow and solid organ transplant recipients. These highly immunogenic lymphomas arising in immunocompromised hosts express all latent EBV antigens (latency type III), including immunodominant EBV nuclear antigen (EBNA)-3A, -3B, and -3C, and are therefore ideal targets for immunotherapy. By contrast, NPC only express a restricted set of less immunogenic viral antigens (latency type II), namely EBNA-1, and LMP-1 and LMP-2. EBNA-1 is expressed regularly in NPC. Although its processing through the HLA class I pathway is inhibited by a glycin-alanine repeat and is an unlikely target for $CD8^+$ effectors, it is a dominant target for $CD4^+$ T cells. Expression of LMP-1 and LMP-2 is detectable in at least 50% of NPC tumors. LMP-1 and LMP-2 are both targets for $CD8^+$ CTL. Responses detected in healthy virus carriers indicate that LMP-1 is poorly immunogenic, thus the most likely target antigen for a $CD8^+$ CTL-based therapy is LMP-2.[84–86]

The pilot study using adoptive T cell therapy to treat NPC was reported in 2001.[87] Autologous EBV-transformed B-lymphoblastoid cell line (LCL) reactivated T cells were generated *in vitro* and used to treat four advanced cases of NPC. The use of autologous EBV-specific CTL for NPC has since been evaluated in two clinical trials.[88,89] Both studies demonstrated that autologous EBV-specific CTL is safe, induces LMP-2-specific immune responses, and is associated with the objective response and control of disease in advanced NPC. Interestingly, Comoli *et al*. also reported that the adoptive transfer of an allogeneic EBV-specific CTL in one patient with relapsed NPC resulted in temporary stabilization of disease.[89] Local tumor biopsy showed an increase in tumor-infiltrating CD8 T cells.[90] However, in these studies the EBV-specific CTL lines were generated by stimulation with EBV-LCL, which favored the outgrowth of CTL responses to the immunodominant EBNA-3 proteins rather than the subdominant EBV proteins LMP-1 and LMP-2 expressed in NPC. Antitumor response could be further enhanced by strategies that increase the specificities of CTL lines for the EBV latency II antigens expressed in NPC.[91–94] A vaccine consisting of dendritic cells pulsed with peptides derived from LMP-2 has been evaluated in 16 NPC patients with local recurrence or distant metastasis after conventional treatment.[95] Peptide-specific T cell responses were elicited or boosted in nine patients and partial tumor reduction was observed in two patients. Currently, a vaccination trial is ongoing in the UK and our center using a modified vaccinia virus expressing an EBNA-1–LMP-2 fusion protein to elicit $CD4^+$ and $CD8^+$ T cells against the two EBV proteins expressed in NPC patients.[91] Alternatively, a LMP-based polyepitope vaccine has also been developed for EBV-associated Hodgkin disease and NPC.[92,93]

Conclusions

In summary, systemic chemotherapy has become an integral part of the clinical management of NPC. In the curative setting, the optimal sequencing of adjunctive chemotherapy and chemoradiation, and the clinical application of the biomarker plasma EBV DNA in determining treatment decisions on adjuvant chemotherapy, represent the two most important clinical questions. The use of molecular targeted agents is still confined to the phase I and II levels of development in the metastatic and recurrent settings, and greater effort should be spent on defining their role in retarding cell metastasis, and in prolonging disease remission after palliative chemotherapy. With myriad promising molecular targets for NPC that are currently being tested, one should also look for predictive biomarkers of response for these agents in order to rationalize patient selection for clinical trials. Vaccines provide a potential application as an effective adjuvant therapy in lowering the risk of recurrence after chemoradiation. Ultimately, the mission of clinical researchers is to find the best way of applying these new strategies into clinical practice, and in this regard one cannot overemphasize the importance of enlisting multicenter or multinational collaboration in the phase III validation of promising therapies.

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