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Review

# Nasopharyngeal carcinoma: The next challenges

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ABSTRACT

Nasopharyngeal carcinoma (NPC) differs from other head and neck cancers in its aetiology, epidemiology and potential therapeutic options. Despite cure for the majority of the patients, challenges still exist in the prevention of disease relapse, treatment of patients with refractory or metastatic NPC and the management of long-term toxicities. This article discusses the specific challenges in pushing the boundaries of NPC treatments further, with an emphasis on prognostic/predictive markers, molecularly targeted therapies, immunotherapies and the areas of interest with regard to long-term toxicities arising from therapeutic interventions.



## 1. Introduction

Nasopharyngeal carcinoma (NPC) is a disease with distinct ethnic and geographic distribution. This tumour is relatively rare in the Western world, but represents a significant disease burden in Southern China and Southeast Asia, with an annual incidence rate of about 20 per 100,000 people in endemic areas.[1,2] Globally, NPC accounts for 80,000 new cases and 50,000 deaths annually.[2]

The aetiology of NPC is complex, and includes a host of viral, genetic and environmental factors.[3–6] It is widely accepted that Epstein-Barr virus (EBV) infection plays a major role in the pathogenesis of NPC in both endemic and non-endemic areas.[7–9] The association between NPC and EBV was initially discovered from serological studies and was later supported by demonstration of EBV DNA and nuclear antigen proteins (EBNA) in NPC tumour cells.[7,10] The notion that EBV plays an important role in the development of NPC is further supported by the observation that early nasopharyngeal lesions (dysplasia or carcinoma-*in situ*) are already EBV-positive, harbouring latent and clonal viral genomes as well as viral oncoproteins such as latent membrane protein (LMP) 1.[8]

The majority (75–90%) of newly diagnosed NPC patients have loco-regionally advanced disease, commonly with cervical nodal metastases.[11,12] Currently, the standard of care for these patients consists of concurrent chemo-radiotherapy with cisplatin-based regimens, generally followed by adjuvant chemotherapy. This treatment approach results in cure for the vast majority of patients, with 3-year disease-free and overall survival rates of approximately 70% and 80%, respectively.[13,14] Recent data demonstrated that 15–19% of all NPC patients fail

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with distant metastases while contemporary results using intensity-modulated radiotherapy (IMRT) rarely yield any local or regional lymph node recurrences.[15–17] These results clearly indicate that NPC is no longer a problematic disease from a loco-regional control standpoint and research priorities should lie in the development of innovative strategies in order to prevent distant relapse, prolonging remission in those with metastatic disease and minimising treatment toxicities. A key area for improvement in the management of the loco-regional setting of NPC is to maintain excellent disease control while incorporating novel therapeutic strategies that can potentially minimise toxicity. The increased understanding of the hallmarks of cancer and advances in biotechnology has led to the development of molecularly targeted therapeutics. Given the relevance of EBV in NPC pathogenesis, as well as the myriad of molecular aberrations secondary to EBV, the concentration of research efforts that directly or indirectly interrogate this viral target seems logical.

In this review, we aim to examine key therapeutic challenges in NPC that warrant continual research and evaluation, with particular emphases on prognostic/predictive markers, molecularly targeted therapies and immunotherapies as possible strategies beyond conventional cytotoxic regimens. Lastly, issues relevant to the prevention and management of long-term treatment toxicities in NPC patients are discussed.

## 2. Prognostic and predictive biomarkers

There is a significant need for early identification of patients who are at risk of relapse after their primary treatment. In doing so, new treatment strategies including upfront use of novel agents as well the use of adjunct therapies could be developed and assessed. In this section, we will discuss potential bio/imaging markers that may identify patients who at the greatest risk of relapse.

### 2.1. *EBV DNA and antibody titres*

EBV not only plays a role in the aetiology of this disease, but its status has prognostic implications. It has been demonstrated that high pre-treatment EBV DNA load, likely representing a greater tumour burden in the patient, is associated with advanced disease stage and serves as an independent risk factor for decreased survival compared to the other parameters that comprise the conventional anatomical stage classification.[18]

A recent study assessed the prognostic value of plasma EBV DNA concentrations in patients with advanced NPC. In this study, 99 patients were treated with neoadjuvant cisplatin-based chemotherapy followed by radiotherapy (RT). Plasma EBV DNA titres were assessed prior to treatment, on days 35 and 64 during neoadjuvant chemotherapy, and one week after completing RT. At baseline, 94 of 99 patients had detectable plasma EBV DNA while levels were undetectable in healthy controls. EBV DNA concentrations were persistently low or undetectable in patients with clinical remission, while these levels were significantly higher in patients with relapsed disease. Higher pre-treatment EBV DNA corresponded to a decreased disease-free survival and overall survival.[19] These findings are further supported by another study that demonstrated a high level of pre-treatment EBV DNA in NPC patients is associated with a higher risk of disease recurrence and disease-related death.[20]

Antibodies against EBV viral capsid antigen (VCA) have also been shown to have prognostic value. In one study with 75 patients, it was shown that IgA EBV VCA titres had a prognostic value on overall survival while IgG titres were prognostic for local control of disease.[21] This observation, however, was not replicated in another study involving 114 patients where pre-treatment antibody levels of both IgG and IgA were not predictive for relapse.[22] In the latter study, it was demonstrated that plasma EBV DNA was a superior prognostic tool compared to EBV VCA antibodies.

We believe that the use of EBV DNA titres pre and post treatment could be useful in the clinical setting and trials that evaluate novel strategies for patients with persistently detectable titres post treatment are warranted. At present there is an ongoing study, investigating the role of gemcitabine and cisplatin doublet as adjunct treatment in NPC patients with detectable EBV DNA post primary RT.[23]

### 2.2. *Excision repair cross-complement (ERCC)*

The excision repair cross-complementing (ERCC) 1 enzyme plays a crucial role in the nucleotide excision repair (NER) pathway. It has been hypothesised that in tumour cells treated with cisplatin, impaired function of the NER pathway (including lack of ERCC1) would lead to a greater sensitivity to platinum-induced DNA damage with subsequent cell death.[24]

In non-small cell lung cancer, low ERCC1 mRNA expression is predictive of response to platinum-based therapy in the advanced setting while low protein expression has been shown to predict benefit for adjuvant cisplatin-based adjuvant chemotherapy.[25,26] ERCC1 therefore is of particular interest in NPC patients as primary treatment regimens often contain platinum-based chemotherapy. In two separate studies, single nuclear polymorphism (SNP) in codon 118 on the *ERCC1* gene demonstrated a predictive value for response to oxalipatin-based chemotherapy in a colon cancer population.[27] The evaluation of this SNP has been demonstrated to be feasible in NPC patients, warranting further investigation.[28] Although this particular study by Ma et al. did not demonstrate a predictive value codon 118 SNP of the *ERCC1* gene, this trial population was quite small, hence might not be powered to detect such differences.

### 2.3. *[18]Fluorodeoxyglucose Positron Emission Tomography (FDG PET)*

In tumours such as advanced gastrointestinal stromal tumours (GISTs), FDG PET scans have been considered to be a useful tool to assess early tumour response to treatment.[29] Functional imaging is still largely an unexplored area in NPC, but a recent study suggested that a high standardised uptake value (SUV) on FDG PET scans have a potential utility as a prognostic marker.[30] In this study, 41 non-disseminated NPC patients underwent FDG PET evaluation pre and post radio(chemo)therapy. Patients having tumours with high pre-treatment SUV had a significantly lower 3-year disease-free survival than patients with

lower FDG uptake (51% versus 91%, $p = 0.007$).[30] However, in another study ($n = 39$), the use of pre-treatment FDG PET scanning did not demonstrate a prognostic value but its use was informative in predicting local response post completion of treatment.[31] Indeed, a meta-analysis has also shown that FDG PET scan is the best modality for diagnosis of local residual or recurrent nasopharyngeal carcinoma.[32] These preliminary results demonstrated the potential use of FDG PET scans for predictive and possibly prognostication purposes.

## 3. Translating bench to bedside

Preclinical research has contributed vastly to our current knowledge pool in cancer science. However, problems often exist especially in translating the knowledge gained from the laboratory setting into clinical trials. In cell culture experiments including work involving NPC, there is growing realisation that cell lines can be cross-contaminated or misidentified, resulting in the dissemination of erroneous information.[33–38]

In NPC, cell culture work also has its own unique challenges. Despite the viral aetiology of this disease, there are only a few NPC cell lines, such as C666-1, which stably harbour the EBV genome and can thus be used as EBV-positive NPC model.[39] In addition, there is also a scarcity of cell lines derived from metastatic or treatment-refractory NPC which are relevant as this represents the patient cohort with a limited prognosis in clinical practice.

One of the main steps to remedy the problem of cell line misidentification is by achieving a consensus on appropriate cell line work reporting. This includes the authentication of cell lines by short tandem repeat (STR) studies that utilises DNA signature profiling technology.[40,41] Since this type of tool is now readily available, it should be applied rigorously and routinely when cell line work is reported. To stipulate and standardise this practice, some journals require details to be provided on cell line lineage and authentication for reports that are being considered for publication.[42]

## 4. Molecularly targeted therapy in NPC

There is a host of molecular, genetic and epigenetic aberrations documented in NPC (Table 1),[43–59] many of which represent a direct or indirect result of EBV infection (Fig. 1). The identification of these aberrations has logically led to the assessment of molecularly targeted therapies in this disease. In NPC, the two most investigated molecularly targeted therapies revolve around the inhibition of epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR). In this segment, we will discuss current data as they pertain to these two classes of molecules (Table 2), as well as the modulation of other important aberrant tumourigenic pathways in NPC.

### 4.1. *Receptor-mediated molecular aberrations*

#### 4.1.1. *Epidermal growth factor receptor (EGFR)*

EGFR over-expression has been found in over 80% of NPC cases,[43–45] and is associated with late-stage disease and poor clinical outcome, including decreases in overall and disease-free survival.[50,60,61] It has also been demonstrated that the EBV-encoded LMP1 activates NFκβ and STAT3, leading to increased BCL-3 with resultant up-regulation of EGFR.[62–65] These findings render targeting EGFR a logical and attractive strategy in the treatment of NPC.

Despite these biological observations, however, the use of anti-EGFR therapy in the recurrent or metastatic disease setting has been largely disappointing. Two phase II trials utilising the EGFR tyrosine kinase inhibitor gefitinib as a single agent have reported no evidence of objective response, while stable disease rates ranging from 11% to 19%.[66,67] In a phase II combination trial of cetuximab (an antibody against EGFR) and carboplatin in patients who have progressed on or within 12 months after termination of platinum-based chemotherapy for recurrent or metastatic NPC, Chan et al. reported more promising results with an overall response rate (ORR) of 12% and a stable disease rate of 48%.[68] In another trial, the role of maintenance therapy with the EGFR tyrosine kinase inhibitor erlotinib after six cycles of platinum-gemcitabine chemotherapy has recently been presented.[69] In this phase II study, erlotinib was found to be ineffective as a maintenance therapy with the majority of patients progressed on first assessment whilst on treatment.

In the non-metastatic NPC setting, there has been a phase II trial assessing platinum-based chemo-radiotherapy and cetuximab. Preliminary efficacy data from this study which consisted of 12 evaluable patients demonstrated a complete response in 10 patients while the remainder had partial response.[70] Recently, investigators at Sun Yat-Sen University also completed a multicentre phase II clinical trial that accrued 100 patients on whom results are awaited. The ENCORE study combined cetuximab with IMRT and concurrent cisplatin chemotherapy in loco-regionally advanced nasopharyngeal carcinoma.[71] The envisioned strategy exploited the survival benefit that was evident in squamous cell head and neck cancers (SCCHN) using cetuximab combined with RT alone reported by Bonner et al.,[72,73] and to combine this with conventional treatment of NPC. Shortcomings of this approach in NPC include the fact that contemporary management with IMRT and chemotherapy only very rarely results in loco-regional failure while distant metastasis remains the formidable challenge.[17] In contrast, Bonner et al. achieved the survival advantage in SCCHN through enhancement of loco-regional control by augmenting the effect of RT alone without increase in the in-field toxicity, but had no impact on distant metastasis. Potentially, a therapeutic window could exist for NPC by permitting IMRT dose with cisplatin to be reduced, with obvious local toxicity benefit, when combined with cetuximab since the contemporary reported loco-regional efficacy is so exceptional with IMRT doses of 70 Gy. At the same time it is conceivable that the combination of cetuximab with cisplatin could be advantageous in addressing distant metastasis. Proof of principle for a synergistic effect may be derived from the EXTREME trial reported by Vermorken et al. that showed a survival advantage for patients treated with cetuximab, 5-fluorouracil and platinum triplet combination compared to 5-fluorouracil-platinum doublet in the recurrent or metastatic SCCHN setting and that was not mediated by enhancement of RT effect.[74] These principles require reliance at some level that the observations in SCCHN can be extrapolated to NPC.

**Table 1 – Molecular aberrations in NPC.**

| Mechanism | Pathway | Aberration | Frequency (%) | References |
|---|---|---|---|---|
| Receptor-mediated | EGFR | Protein expression | 83 | [43–45] |
| | VEGF | mRNA over-expression | 40 | [46] |
| | | Protein expression | 46–67 | [47,48] |
| | c-MET | Protein expression | 52 | [55] |
| | c-Kit | Protein expression | 33 | [56] |
| | Her-2 | Protein expression | 0–33 | [49,56] |
| Intra-cellular mitogenic signals | PI3K | Amplification of *PIK3CA* | 22 | [53] |
| | | Mutation of *PIK3CA* | 1–10 | [51,53] |
| | AKT | Protein expression | 36–42 | [58] |
| | HIF-1α | Protein expression | 32–58 | [57,59] |
| Cell cycle | Cyclin-D1 | Gene over-expression | 92 | [54] |
| | | Protein expression | 20 | [54] |
| Genetic/epigenetic | Gene deletion and hypermethylation | P16/CDKN2A | 62–86 | [43] |
| | | P14/ARF | 54 | [43] |

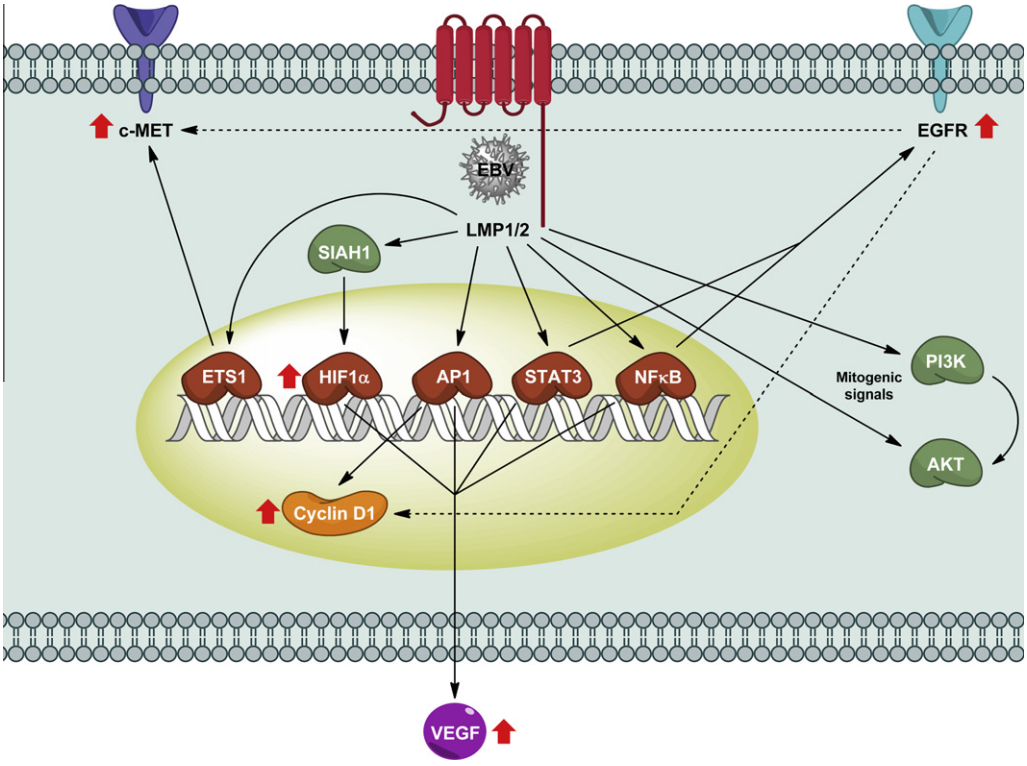


**Fig. 1 – Molecular aberrations in NPC arising from EBV infection. AKT – AKT protein family (also known as protein kinase B), AP-1 – activator protein 1, c-MET – mesenchymal epithelial transition factor, cyclin-D1 – cyclin protein D1, EBV – Epstein-Barr virus, EGFR – epidermal growth factor receptor, ETS1 – ETS1 family protein, HIF-1α – hypoxia inducible factor 1 alpha, LMP – latent membrane protein, NFκβ – nuclear factor kappa light chain enhancer of activated B cells, PI3K – phosphoinositide 3-kinase, SIAH1 – seven in absentia homologue 1, STAT 3 – signal transducer and activator of transcription 3 and VEGF – vascular endothelial growth factor.**

There are several additional challenges in determining the value of anti-EGFR therapy in NPC. First, the results of trials in recurrent or metastatic NPC are difficult to gauge in terms of efficacy, largely due to heterogeneity of trial populations enrolled. Second, unlike the use of EGFR tyrosine kinase inhibitors in non-small cell lung cancer, there has been no identification of predictive factors for efficacy (such as EGFR mutations and/or increased EGFR copy number), which perhaps explains the disappointing results to date.[75–77] Third, as the assessments of anti-EGFR therapy have been largely disappointing, it is important to ascertain the role of downstream signalling inhibition of this pathway, or alternate pathways, which may account for resistance to EGFR inhibitors and be of greater therapeutic relevance in NPC. Initial

**Table 2 – Summary of key trials of molecularly targeted therapy in nasopharyngeal cancer.**

| Agent | Phase | Treatment | N | ORR (%) | CR (%) | SD (%) | TTP (mo) | PFS (mo) | OS (mo) | REF |
|---|---|---|---|---|---|---|---|---|---|---|
| *Single or combination therapy in metastatic/recurrent setting* | | | | | | | | | | |
| Gefitinib | II | Gefitinib 250 mg daily | 19 | 0 | 0 | 10.5 | 4 | – | 16 | 66 |
| | II | Gefitinib 500 mg daily | 16 (15 evaluable) | 0 | 0 | 18.8 | 2.7 | – | 12 | 67 |
| Cetuximab | II | Cetuximab 250 mg/m$^2$ weekly (400 mg/m$^2$ loading dose) + carboplatin | 60 | 11.7 | 0 | 48.3 | 2.7 | – | 7.8 | 68 |
| Erlotinib | II | Cisplatin/carboplatin + gemcitabine followed by erlotinib (E) maintenance 150 mg daily | 20 (11 evaluable for E) | 0 | 0 | 27 (on E) | 6.3 (evaluable pts) | – | – | 69 |
| Sorafenib | II | Sorafenib 400 mg bid daily | 7 | NR | NR | NR | 3.2 | – | 7.7 | 82 |
| *Concurrent therapy in locally advanced disease* | | | | | | | | | | |
| Cetuximab | II | IMRT + cisplatin + cetuximab 250 mg/m$^2$/week (loading dose of 400 mg/m$^2$ 7–10 d prior to RT) | 20 (12 evaluable) | 100 | 83 | – | – | – | – | 70 |

NR = not reported.

data in this area thus far are disappointing as known downstream activating mutations which may account for EGFR resistance, such as *RAF* and *RAS*, have been shown to be absent in NPC.[51] Lastly, combination therapy, especially with conventional radiation doses, seems to yield a high level of toxicity, with little information of short and long-term sequelae.[78,79] The validation of EGFR as a therapeutic target and proof that its inhibition optimises the therapeutic index in NPC are critically important issues which need to be addressed in this malignancy.

#### 4.1.2. *Vascular endothelial growth factor (VEGF)*

There is good rationale for the use of anti-angiogenic therapy in NPC that blocks VEGF or its receptor (VEGFR) utilising agents such as the monoclonal antibody bevacizumab or the small molecule tyrosine kinase inhibitor sorafenib, respectively. VEGF over-expression has been demonstrated in 40–70% of NPC patients and its presence is associated with a higher rate of metastatic relapse and a worse overall survival.[46–48] EBV viral latent/nuclear antigens bind to promoter sites of c-Jun and ATF2 resulting in an enhanced activity of the AP-1 transcription factor, which in turn leads to VEGF up-regulation.[80,81] VEGF over-expression has also been shown to be a result of NFκβ related pathways,[80] rendering the inhibition VEGF an attractive target.

Despite these findings, there is a dearth of trials assessing the role of anti-angiogenic therapy in NPC. In one phase II trial, seven patients with recurrent or metastatic NPC were treated with sorafenib; the median time to treatment progression was 3.2 months with an associated overall survival of 7.7 months.[82] One of the original hypotheses supporting the use of anti-angiogenic therapy in loco-regionally advanced NPC was the reported association of VEGF expression with angiogenesis and lymph node metastasis and a putative consequent risk of distant metastasis.[83] To address this hypothesis, the Radiation Therapy Oncology Group (RTOG) recently completed accrual to a phase II trial (RTOG 0615) assessing the role of bevacizumab added to concurrent chemo-radiotherapy and adjuvant cisplatin and 5-fluorouracil in loco-regionally advanced NPC.[84] Anti-angiogenic agents do not yet have proven biological activity in NPC, thus the challenges with incorporating them in the treatment of NPC invoke similar considerations as previously outlined for anti-EGFR therapies. In light of the negative results of bevacizumab given with adjuvant chemotherapy in resected colon cancer,[85] and in combination with cetuximab plus chemotherapy in metastatic colon cancer, the role of anti-angiogenic therapy in the treatment armamentarium of NPC is uncertain and will need further evaluation.[86]

#### 4.1.3. *Mesenchymal-epithelial transition factor (c-MET)*

The receptor tyrosine kinase c-MET is the cell surface receptor for hepatocyte growth factor.[87] The activation of this receptor in cancer correlates with poor prognosis, where aberrantly active MET triggers tumour growth, angiogenesis and metastasis.[87] In NPC patients, MET protein expression is present in 52–72% of patients, associated with cervical nodal metastases and poor prognosis.[55,88] It is thought that LMP1 could cause over-expression of c-MET by induction of transcription factor Ets1.

There is also *in vitro* evidence suggesting cross-talk between the c-MET and EGFR pathways wherein EGFR activation can phosphorylate and activate c-MET.[89] At present, there are c-MET inhibitors undergoing clinical development and their use serves as attractive therapeutic candidates in NPC, potentially in combination with anti-EGFR therapy.

### 4.2. *Intracellular mitogenic signals*

#### 4.2.1. *PI3K/AKT/mTOR inhibition*

The P13K/AKT/mTOR pathway regulates several critical cellular functions including proliferation, growth, survival and mobility. In NPC, activation of PI3K and AKT pathways is present in 21–75% of cases and may be associated with poorer outcome.[51–53] It has been shown that the EBV LMP 1 and 2 activate the PI3K/AKT pathway.[90] Inhibition of this cascade has become viable as there are currently P13K and AKT inhibitors under development while mTOR inhibitors such as everolimus have already established therapeutic roles in renal cell carcinoma.[91] *In vitro* data thus far have demonstrated that AKT and mTOR blockade inhibits cell growth in a variety of NPC cell lines in nanomolar concentrations, including those which harbour EBV.[92,93] There is currently no known clinical trial for NPC patients utilising these agents.

#### 4.2.2. *Hypoxia inducible factor 1 alpha (HIF-1α)*

Triggered by tumour hypoxia, the transcriptional factor HIF-1α is activated and leads to up-regulation of several genes associated with evasion of apoptosis and angiogenesis such as carbonic anhydrase and VEGF/VEGFR respectively.[59,94,95] It has been shown that in NPC, the expression of HIF-1α is associated with poorer survival and increased occurrence of tumour invasion and distant metastases.[95] There is also evidence that the expression of LMP1, as a result of EBV infection induces HIF-1α expression via Siah1-mediated (ligase involved in ubiquitination and degradation of other specific proteins) mechanism.[96,97] HIF-1α expression is also positively correlated with up-regulation of VEGF expression. At present, there are several inhibitors of HIF-1α in development. Given the molecular associations above, the inhibition of this pathway warrants further evaluation.

### 4.3. *Cell cycle aberrations*

#### 4.3.1. *Cyclin-dependent kinases (CDKs)*

CDKs are serine/threonine kinases that play important roles in cell cycle regulation. Their activity is controlled through a highly regulated process with positive regulation provided by regulatory subunits such as cyclins, while naturally occurring CDK inhibitors (CDKi) have a negative regulatory effect.[98]

In NPC, cell cycle dysregulation has been well documented, in which cyclins such as cyclin-D1 are often over-expressed while CDKi such as $p16^{INK4A}$ are down-regulated.[54,99] This could in part be explained by LMP1 inducing the transcription factor c-Jun/Jun B heterodimer and methylation of the p16 promoter gene, resulting in over-expression of cyclin-D1 and down-regulation of p16, respectively.[100]

These events result in the facilitation of G1 cell cycle progression through phosphorylation of the retinoblastoma protein, thereby promoting E2F-mediated gene transcription. Restoration of p16 expression[101] or inhibition of cyclin-D1 by small interfering RNA reduces proliferation of NPC cell lines, providing rationale for CDKi therapy.

Seliciclib is an oral small molecule CDKi with known activity against anti-apoptotic proteins such as cyclin-D1.[64] *In vitro* data demonstrated evidence of apoptosis and cell cycle arrest, as well as synergism with RT when seliciclib was used in an EBV-harbouring NPC cell line.[102] The use of this agent was also associated with enhanced apoptosis in *in vivo* models.[102] In one early clinical trial, the use of seliciclib in 16 treatment naïve NPC patients resulted in stable disease in all evaluable patients ($n = 14$, seven with tumour reduction).[103] Common adverse events included low-grade fatigue, nausea, vomiting, constipation, cough, fever, hypokalemia, hyponatremia and elevation in transaminases. Further evaluation of this agent in previously treated NPC patients is currently ongoing.[104]

### 4.4. *Epigenetic aberrations*

Many genetic and epigenetic changes have been demonstrated in the development and progression of NPC. Studies have revealed a high incidence of loss on chromosomes 3p (75%), 11q (70%) and 14q (65%).[6,43] Losses of 9q (60%), 13q (50%) and 16q (40%) were also identified.[6,43] Novel chromosomal gains were observed on chromosome 12, with a high frequency (70%).[105,106]

#### 4.4.1. *Methylation of promoter regions*

In addition to these chromosomal abnormalities, there is also evidence of frequent promoter hypermethylation of tumour suppressor genes such as 9p21 (p14, p16) and 3p21.3 (RASSF1A).[105,107] Aberrant promoter methylation has been demonstrated to occur in other cancer related genes such as TSLC1, RARß2 and E-cadherin.[108–110] The aberrant promoter methylation of these genes results in disruption of multiple cellular functions through the inactivation of retinoid signalling pathway (e.g. RARB2) and endothelin-cell adhesion (e.g. E-cadherin, TSLC-1). Furthermore, there is also evidence that methylation of the promoter regions of EBV latent and lytic antigens are implicated in the epigenetic silencing of the virus in NPC, facilitating avoidance from immune surveillance by cytotoxic T-cells.[94,111]

Given these abnormalities, the utilisation of hypomethylating agents poses an attractive treatment platform for NPC patients. Currently, there are several hypomethylating agents in development or available, such as decitabine and azacitidine. These function as cytosine/cytadine analogues and inhibit the activity of DNA methyltransferases. Early data indicate that the use of azacitadine in NPC patients results in substantial demethylation of many latent and lytic EBV promoter regions.[112]

#### 4.4.2. *Histone deacetylation*

Histone deacetylation is another important epigenetic process potentially relevant in NPC. Chromatin modulation by histone acetyltransferases and histone deacetylases (HDACs) represents an integral mechanism for the regulation of gene transcription. Since HDACs have been mechanistically linked to the pathogenesis of human malignancies, and inhibitors of

HDAC (HDACi) have demonstrated *in vitro* activity in NPC, this treatment avenue warrants further investigation.[113,114] It has been postulated that reversal of both promoter methylation and histone deacetylation might be superior to the reversal of only one mechanism[115]; hence the combination of HDACi and hypomethylating agents serves as an attractive therapeutic strategy which is currently being examined in a clinical trial assessing azacitadine and vorinostat (a HDACi) in relapsed and/or metastatic patients with NPC.[94,116]

## 5. Immunotherapy

Despite its infectious aetiology, it is recognised that EBV infection is associated with immune escape mechanisms, whereby EBV establishes a state of persistent infection through its latency in memory B cells. While the virus expresses numerous lytic antigens (>80), there are three latent EBV proteins expressed in NPC (EBNA1, LMP 1 and 2).[117–120] In addition to the relatively diminished viral protein expression, EBV has further latency functions with non-translated RNAs that cannot be detected by T-cells.[120] Furthermore, EBV infection also seems to induce local immune suppression and is associated with a low level of circulating T lymphocytes when the EBV viral burden is increased.[121–123] These findings have provided an attractive rationale for investigating immunotherapeutic approaches in fighting this disease.

### 5.1. *Adoptive immunotherapy*

The expression of LMP1 and 2 has triggered adoptive immunotherapy approaches utilising cytotoxic T lymphocytes (CTL). The feasibility of utilising autologous EBV-specific CTLs in NPC has been demonstrated in at least three early phase studies. In one study, the use of EBV-specific CTL was assessed in 10 NPC patients (four in disease remission while the remainder had refractory disease). All four patients who were in remission remained disease-free 19–27 months after infusion, while in patients who had refractory disease, two had complete responses and two others had a partial response and stable disease, respectively.[124] The treatment was well tolerated apart from swelling at a pre-existing disease site in one patient. In another study, 10 patients with documented progression of advanced stage NPC were treated with EBV-specific CTLs. Disease control was achieved in six patients, with two partial responses while four patients had documented stable disease.[125] Again, the use of CTL in this setting was safe and tolerable apart from grade 1/2 inflammation in known disease sites. The feasibility and safety of this approach were further demonstrated in a third study involving five evaluable patients with advanced, pre-treated patients and resulted in stable disease in one patient.[126] There is at present a phase I study ongoing evaluating LMP1 and 2 specific CTLs given with CD45 monoclonal antibody (the latter given to cause lymphodepletion) in patients with EBV-positive NPC.[127]

### 5.2. *Active immunotherapy*

The viral aetiology of NPC has also led to the evaluation of active immunotherapy in this disease. In one study, 16 patients with disease recurrence after conventional therapies, received EBV vaccine therapy pulsed with three different epitope peptides. Epitope-specific cytotoxicity was detectable in vaccination responsive patients, and in two patients, this coincided with tumour reduction.[128] Approaches leading to stronger and more sustained EBV-specific T-cell responses may have therapeutic potential in NPC. Currently, there are several vaccine studies ongoing, evaluating this strategy further either as an adjuvant therapy or as active treatment in metastatic setting.[129–131]

## 6. Managing long-term toxicities

A significant number of NPC patients are cured from their disease, but long-term toxicities exist such as xerostomia, soft tissue fibrosis as well as sensorineural hearing loss.[132,133] With the advent of IMRT, the incidence for some of these problems such as xerostomia has been reduced, but many of the long-term toxicities are not well studied or managed.[134]

Late auditory toxicity (more than grade 3) affects up to 27% of NPC.[135] In one study, the use of IMRT does not seem to improve auditory toxicity in NPC patients.[134] It was demonstrated that increasing age, use of chemoradiation (as opposed to RT alone) and the mean RT dose affecting the cochlear (hazard ratio of 1.03 per 1 Gy increase) play significant roles in the development of auditory toxicity.[135]

Temporal lobe necrosis is also a recognised late sequela of RT in NPC. Traditionally two-dimensional RT was the only means of RT delivery and higher doses per fraction were often administered in large centres where significant patient volumes necessitated economically derived regimes that use fewer fractions but are now known to have significant risk. Thus patients with early T1 disease treated with RT alone with 4.2 Gy per fraction regimens had a 10-year actuarial risk of 18.6% of temporal lobe necrosis, despite a total dose limited to 50.4 Gy. Even 2.5 Gy per fraction treatments to a total dose of 60 Gy showed a risk of 4.6%.[136] Partly for these reasons, such RT dose fractionation approaches and RT techniques are now generally considered obsolete in the treatment of NPC. In contrast, the same group recently outlined more recent data that addressed all curative stages of disease and almost entirely comprised patients accrued to two randomised controlled trials in Hong Kong.[135] These trials addressed the respective roles of conformal RT with or without chemotherapy or accelerated fractionation, and the toxicity results portray a different risk profile compared to the historic findings. Lee et al. showed that no patients ($n = 171$) who received standard or accelerated RT at 70 Gy with 2 Gy per fraction experienced temporal lobe necrosis, provided no chemotherapy was used; as noted the decision to use or omit chemotherapy was based on randomised trial allocation. If an additional brachytherapy boost confined to the soft tissues of the nasopharynx was used, there also were no patients with temporal node necrosis. However when an external beam boost was used that delivered an additional dose of 5 Gy in two fractions above the principal dose of 70 Gy in 35 fractions, the incidence of temporal lobe necrosis increased to 8.3% at 5 years. When chemotherapy was used ($n = 196$) in the 70 Gy in 35 fractions regimen, a mild emergence of temporal lobe necrosis occurred (1.3%) but was entirely confined to the 101 patients who also received accelerated fractionation (2.7% risk). In Lee et al.'s recent data, there were

no cases of necrosis in 264 temporal lobes treated to a mean dose of ⩽22 Gy compared to a 2.2% rate in 128 temporal lobes treated with greater doses.[135] While many of their patients were treated with conformal RT rather than two-dimensional techniques, it is likely, though as yet unproven, that IMRT could virtually relegate the problem to the past since these dose objectives to the temporal lobe are readily achievable. There is currently no consensus of how temporal lobe necrosis should be managed and it seems prevention may be the best strategy. Of note, recent publications have reported on the use of anti-VEGF therapy in radiation-induced brain necrosis with promising results.[137,138]

Another increasingly recognised area of treatment-related complication is neurocognitive impairment. Neurocognitive impairment is a recognised treatment toxicity in a number of tumour sites such as breast and brain cancers.[139–142] There are scant data suggesting the same effects apply in head and neck patients.[143,144] In one study involving 30 NPC patients, it was demonstrated that up to 77% of patients had significantly lower neurocognitive assessment scores post treatment ($p = 0.03$).[144] Neurocognitive areas affected include short-term memory, language abilities and list generating fluencies. The neurocognitive decline was shown to be significant when the mean dose of temporal lobe radiation exceeded 36 Gy or when the V60 (volume receiving 60 Gy or more) of the temporal lobes exceeded 10%.[144]

These long-term problems have not been comprehensively studied in NPC patients and further research is warranted, to provide guidance on the optimal management of such adverse effects and on effective strategies that may prevent or reduce such complications.

## 7. Conclusion

In light of the evolving natural history of NPC, there remain significant challenges in developing effective and meaningful therapeutic strategies in this disease. The areas of challenges include improvement in the bench to bed discoveries, coupled with exploitation of the knowledge gained from important genetic and epigenetic pathways in this disease leading to novel therapeutic strategies. Targeted therapy aimed at aetiology (immune-based therapy) or correction of selected tumourigenic pathways will be crucial in pushing the boundaries further. There is also a great need to understand and explore prognostic and predictive biomarkers in NPC patients, so that patients at risk for an inferior outcome could be offered novel and/or adjunct treatment. The prognostic markers could also serve a function to select patients with particularly good prognosis, so that treatment 'de-intensification' could be considered. Lastly, as there are a significant number of patients who would have successfully combated this disease, but living with long-term toxicities of treatment, particular attention on managing these treatment-related adverse effects in NPC survivors would be of importance.

## Conflict of interest statement

L.L. Siu has received research funding from Bayer, Pfizer, Roche and Cyclacel. Albiruni R.A. Razak, Fei-Fei Liu, Emma Ito, Brian O'Sullivan and Kelvin Chan have no conflict of interest to declare.

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