Back to the drawing board

Resistance and its implications for the use of targeted agents in head and neck squamous cell cancer

Kara Ruicci

Faculty Reviewers: Anthony Nichols, MD, FACS, FRCSC (Department of Otolaryngology, Head & Neck Surgery) & John Barrett, PhD (Department of Otolaryngology, Head & Neck Surgery)

ABSTRACT

A significant limitation of genomically-guided targeted cancer therapies is the inevitability of innate or evolved resistance. Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous and anatomically complex cancer in which both the disease and available treatments carry considerable toxicity with lasting effects on patient quality of life; therapies with increased efficacy and decreased toxicity are needed. The therapeutic focus for HNSCC is shifting towards targeted inhibition of specific, frequently altered genes or pathways, including the phosphatidylinositol 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) network, which is the most frequently altered pathway in HNSCC. Agents targeting this network have demonstrated preclinical and clinical efficacy; however, as in most solid tumour malignancies, response to therapy is temporary and systems become refractory following prolonged treatment.

In this review, we examine the role of emerged resistance with particular regard to targeted PI3K inhibitors in HNSCC and describe targetable mutations, pathway reactivation and bypass mechanisms as mediators of resistance. We conclude by emphasizing the value of combinatorial therapies and the value in re-evaluation of response to therapy over time to prevent or delay the onset of resistance. PI3K inhibitors and other targeted therapies have already begun transforming cancer care, providing improved patient responses and quality of life benefits. Characterizing resistance mechanisms will help guide the application of such agents, as well as the design of combination treatments to improve outcomes for cancer patients.

INTRODUCTION

Resistance to drug therapy is a major challenge in modern oncology. Outside of hematologic malignancies, prolonged efficacy of targeted agents has been largely disappointing; emerged resistance to single targeted agent treatments is almost universally inevitable.^{1,2} Resistant subclones emerge through 2 mechanisms: (1) innate (intrinsic, or *de novo*) resistance whereby a mutation exists before treatment in a select number of cells, or in a minor sub-population of cells that eventually dominate due to selective pressure; or (2) evolved (acquired) resistance resulting from clonal expansion of newly altered cells.³

Advancements in next-generation DNA/RNA sequencing (NGS) have prompted an explosion of targeted agents, and muta-

tion-directed therapy has already become standard of care for select cancers. While targeted inhibition of frequently mutated or overactive signalling pathways achieves tumour responses in a portion of cases, these responses have not been durable over time, and clinical application of single-agent targeted therapies has shown only modest benefit.⁴ Here we discuss the role of targeted phosphatidylinositol-3-kinase (PI3K) inhibition in head and neck squamous cell carcinoma (HNSCC) and describe mechanisms of resistance to targeted therapies, including targeting key activating mutations and pathway reactivation, before addressing combination therapies as an approach to overcome resistance.

RESISTANCE TO PI3K INHIBITION IN HNSCC

HNSCC is the 5th most common malignancy worldwide with over 550 000 cases diagnosed annually, and is on the rise due to newfound associations with oral human papillomavirus (HPV) infection.^{5,6} Despite efforts to improve outcomes, the 5-year survival rate is only about 50% for advanced disease. Toxicity associated with both the disease and available treatments (chemotherapy, radiation, surgery) is high, often permanently altering functional and aesthetic features of the head and neck, including facial appearance, breathing, speech, and swallowing. This treatment morbidity highlights an urgent need for improved therapeutics with increased efficacy and decreased toxicity.^{5,7,8}

The first targeted therapy to demonstrate survival advantage in HNSCC addresses epidermal growth factor biology, as the epidermal growth factor receptor (EGFR) is overexpressed in more than 90% of HNSCC.⁹ Cetuximab (trade name Erbitux) is an EGFR monoclonal antibody and remains the only Food and Drug Administration (FDA)-approved targeted agent for HNSCC. Together with recent multi-platform genomic analyses of HNSCC, the PI3K-AKT-mammalian target of rapamycin (mTOR) network downstream of EGFR is emerging as perhaps one of the most critical pathways in HNSCC. Agents targeting this pathway are at various stages of preclinical and clinical development.^{10,11}

The PI3K-AKT-mTOR axis is mutated in 34% of HPV-negative and 56% of HPV-positive tumours and is the only truly targetable pathway in HNSCC, a disease dominated by alterations in tumour suppressors.^{10,12} Alterations to PI3K pathway members have been shown to induce cell line transformations and tumorigenesis in transgenic mice, demonstrating the oncogenic potential of the PI3K network.¹³⁻¹⁵ Additionally, elevated PI3K signaling following deregulation of upstream mediators (eg, EGFR, FGFR1-3, TSC1-2) or aberrations in downstream effectors (eg, PIK3CA, PIK3R1,

mTORC1-2, PTEN) has been shown to induce cell growth, proliferation, and angiogenesis essential for tumour progression.¹⁴ The prevalence of PI3K pathway alterations and its oncogenic capacity in solid tumours has led to the creation of targeted agents against its major effectors, including the 2-aminothiazole derivative BYL719 (Alpelisib; Novartis).¹⁶

BYL719 is a p110 α -specific PI3K inhibitor equipotent for wild-type and somatic mutant forms of the p110 α catalytic subunit of Class IA PI3Ks.¹⁰ Mutations and amplifications in PIK3CA—which encodes p110 α —is frequently amplified and is characterized by 3 hotspot mutations (E542K, E545K, and H1047R/L). These genetic changes were found to be the predominant positive predictors for BYL719 sensitivity. Also available are panisoform PI3K inhibitors, dual PI3K-mTOR inhibitors, as well as drugs targeting other pathway members such as mTOR and AKT.¹⁰

MUTATIONS MEDIATING RESISTANCE

Mathematical models to describe the evolutionary dynamics of cancers in response to targeted agents emphasize that around 50 unique mutations—innate or acquired—may confer resistance to a given drug. As such, single-agent therapy will eventually fail in all cases, even if the lesion recedes to below clinical detection for months before re-emerging. This explains why tumours can recur after long remission periods.²

Approaches to overcome such resistance may include designing agents with greater potency—for instance, irreversible inhibitors or mutant-selective drugs.¹⁸ Irreversible PI3K inhibitors, such as wortmannin, covalently bind target residues, blocking ATP competition indefinitely. Such inhibitors, however, may impart high toxicity due to off-target effects.^{3,18} With regard to selectivity, most targeted agents—including BYL719—do not specifically inhibit mutant forms of their target. If resistance occurs via a mutation in the original target, it may be effective to replace the drug with a mutant-selective counterpart. For instance, osimertinib targets T790M-mutant EGFR, a primary mediator of resistance for growth factor receptor-targeted agents, such as erlotinib or gefitinib.¹⁰ As our understanding of resistance mechanisms continues to develop, considerations for drug potency and selectivity will become increasingly relevant.

PATHWAY ACTIVATION BY UPSTREAM OR DOWNSTREAM ALTERATIONS

Crosstalk between signalling networks is a considerable challenge in designing drugs with specificity and efficacy. For instance, downstream signalling of PI3K is transmitted via several pathways, including the AKT-mTOR network (involved in cell survival) and the MAPK pathway (growth and proliferation) (Figure).^{10,21} RAF and MEK inhibitors targeting the MAPK network, and mTORC1-2 inhibitors have demonstrated efficacy when prolonged treatment with upstream PI3K inhibitors have resulted in tumour resistance.¹⁹

A second strategy to mitigate single-agent resistance is to target upstream or downstream effectors of the primary target. Mutations acquired during cell divisions and/or in response to prolonged therapy do not necessarily occur in the targeted gene but rather in a gene elsewhere in the pathway.¹⁸ PTEN loss, for instance, was

Figure: The PI3K-AKT-mTOR network and associated signaling pathways. Adapted from Castellano & Downward.²¹

found by sequencing metastatic lesions from a patient treated with BYL719 who achieved a clinical response but eventually became resistant and died shortly thereafter.¹⁷ All lesions refractory to BYL719 showed genetic PTEN alterations not present in the pretreatment tumour, each unique and resulting in loss of PTEN expression.¹⁷ PTEN functions immediately downstream of PI3K, where it acts as a negative regulator, effectively managing network activation. It is evident that alterations in critical effectors up- or downstream of the drug target may be capable of modulating therapy resistance, and alterations to treatment regimens to account for these events may be necessary.

BYPASS MECHANISMS

Increasing evidence supports a role for bypass mechanisms in mediating targeted cancer resistance. This involves pathway reactivation via mediators of a pathway independent of the target of interest.³ An early in vitro study of prolonged BYL719 treatment in HNSCC found that models refractory to p110 α inhibition overexpress AXL, a receptor tyrosine kinase that dimerizes with EGFR, leading to PI3K-independent mTOR activation.¹⁹ In such bypass scenarios, dual inhibition of both pathways (here PI3K and EGFR/AXL) may be a potential strategy to prevent or delay the onset of resistance.

Another bypass-type mechanism takes advantage of the deregulation of cellular metabolism frequent in many cancers. Increased acetyl-CoA production afforded by cancer metabolic reprogramming to favour glycolysis permits increased acetylation of lysine residues in RICTOR, a core component of mTORC2.²⁰ RICTOR activation is maintained as a result, forming an activation loop for mTORC2 leading to its continued signalling activity, even when other components of the growth factor receptor pathways are inhibited. By removing the dependency of mTOR activation on up-

stream signalling, systems effectively become resistant to EGFR, PI3K, or AKT-targeted therapies. Altered cellular metabolism therefore has been shown to not only be a factor mediating cancer progression, but more recently, also an opportunity for drug resistance to develop.

CONCLUSION

As resistance continues to demand consideration in clinical settings, strategies to prevent or delay innate and evolved resistance are being investigated. These include (1) designing more selective, potent inhibitors, including agents active against presently “undruggable” targets (for instance, oncogenic or hyperactive RAS); (2) identifying effective drug combinations to maintain cancer inhibitory properties for a longer period of time; and (3) developing drug dosing regimens with improved efficacy and durability. Already, next-generation inhibitors have been designed with improved tolerability and specificity, and combination therapies of 2 or more agents appear effective; one example is dual inhibition of EGFR and PI3K in BYL719-resistant HNSCC.¹⁹ Thorough investigation of the relative value of sequential versus simultaneous combinatorial therapy and research into the implementation of repeated noninvasive techniques to monitor treatment response and the status of emerging resistance variants will provide answers to some of these questions.¹⁸

The ongoing effort to design more specific and potent inhibitors, in combination with continued validation of potential biomarkers identified by NGS of large-scale patient tumour databanks and characterization of resistance mechanisms to targeted agents, is expected to lead to improved design of clinical trials, and ideally, prolonged clinically relevant patient responses. The genetic diversity and complex anatomical location of head and neck cancers make treatment by conventional means challenging; the focus on personalized treatment of genetically-distinct tumours via targeted therapy holds tremendous treatment potential for HNSCC as well as other cancers. Understanding treatment failure due to resistance is critical for the continued development of such therapies and demands preclinical and clinical focus to overcome.

REFERENCES

1. Bozic I, Nowak MA. Timing and heterogeneity of mutations associated with drug resistance in metastatic cancers. *Proc Natl Acad Sci USA*. 2011 Nov 11;111(45):15964-8.
2. Bozic I, Reiter JG, Allen B, Antal T, Chatterjee K, Shah P, et al. Evolutionary dynamics of cancer in response to targeted combination therapy. *eLife*. 2013 Jun 25;2:1-15.
3. Groenendijk FH, Bernards R. Drug resistance to targeted therapies: déjà vu all over again. *Molecular Oncology*. Elsevier B.V; 2014 Sep 12;8(6):1067-83.
4. Martini M, De Santis MC, Braccini L, Gulluni F, Hirsch E. PI3K/AKT signaling pathway and cancer: an updated review. *Ann Med*. 2014 Sep;46(6):372-83.
5. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA: A Cancer Journal for Clinicians*. 2011 Feb 4;61(2):69-90.
6. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *Journal of Clinical Oncology*. 2011 Nov 8;29(32):4294-301.
7. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 2010 Jul;363(1):24-35.
8. Nichols AC, Dhaliwal SS, Palma DA, Basmaji J, Chapeskie C, Dowthwaite S, et al. Does HPV type affect outcome in oropharyngeal cancer? *J Otolaryngol Head Neck Surg*. 2013 Feb 1;42(9):1.
9. Hansen AR, Siu LL. Epidermal growth factor receptor targeting in head and neck cancer: have we been just skimming the surface? *Journal of Clinical Oncology*. 2013 Mar 25;31:1-3.
10. Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol*. 2013 Feb 12;10(3):143-53.
11. Juric D, et al. Phase I study of the PI3K α inhibitor BYL719, as a single agent in patients with advanced solid tumors (aST). *Ann Oncol*. 2014 Sept 28;25(suppl 4): abstract.
12. Lawrence MS, Sougnez C, Lichtenstein L, Cibulskis K, Lander E, Gabriel SB, et al. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature*. 2015 Jan 28;517(7536):576-82.
13. Carver BS, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer Cell*. 2011 May 17;19(5):575-586.
14. Zhao Y, Adjei AA. The clinical development of MEK inhibitors. *Nat Rev Clin Oncol*. Nature Publishing Group; 2014 May 20;11(7):385-400.
15. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *Journal of Clinical Oncology*. 2010 Feb 18;28(6):1075-83.
16. Fritsch C, Huang A, Chatenay-Rivauday C, Schnell C, Reddy A, Liu M, et al. Characterization of the novel and specific PI3K inhibitor NVP-BYL719 and development of the patient stratification strategy for clinical trials. *Molecular Cancer Therapeutics*. 2014 May 5;13(5):1117-29.
17. Juric D, Castel P, Griffith M, Griffith OL, Won HH, Ellis H, et al. Convergent loss of PTEN leads to clinical resistance to a PI(3)K α inhibitor. *Nature*. Nature Publishing Group; 2015 Nov 17;518(7138):240-4.
18. Gioeli D, editor. *Molecular and translational medicine*. New York: Humana Press; 2011. 211 p.
19. Elkabets M, Pazarentzos E, Juric D, Sheng Q, Pelossof RA, Brook S, et al. AXL mediates resistance to PI3K α inhibition by activating the EGFR/PKC/mTOR axis in head and neck and esophageal squamous cell carcinomas. *Cancer Cell*. Elsevier Inc; 2015 Apr 13;27(4):533-46.
20. Masui K, Tanaka K, Ikegami S, Villa GR, Yang H, Yong WH, et al. Glucose-dependent acetylation of Rictor promotes targeted cancer therapy resistance. *Proc Natl Acad Sci USA*. 2015 Jul 28;112(30):9406-11.
21. Castellano E, Downward J. RAS Interaction with PI3K: more than just another effector pathway. *Genes & Cancer*. 2011 Jul 1;2(3):261-74.